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Sugimoto et al.

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[54] **POLYPEPTIDE POSSESSING CYCLOMALTODEXTRIN GLUCANOTRANSFERASE ACTIVITY**

[75] Inventors: **Toshiyuki Sugimoto; Michio Kubota; Shuzo Sakai,** all of Okayama, Japan

[73] Assignee: **Kabushiki Kaisha Hayashibara Seibutsu Kagaki Kenkyujo,** Okayama, Japan

[21] Appl. No.: **794,347**

[22] Filed: **Nov. 12, 1991**

Related U.S. Application Data

[63] Continuation of Ser. No. 438,933, Nov. 22, 1989, abandoned, which is a continuation of Ser. No. 804,487, Dec. 4, 1985, abandoned.

[51] **Int. Cl.**[5] **C12N 9/10;** C12N 15/54

[52] **U.S. Cl.** **435/193;** 435/252.31; 435/252.33; 435/97; 536/23.3

[58] **Field of Search** 435/97, 193, 252.31, 435/252.33; 536/27, 23.3

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Primary Examiner—Charles L. Patterson, Jr.
Attorney, Agent, or Firm—Browdy and Neimark

[57] **ABSTRACT**

The sequence of cyclomaltodextrin glucanotransferase (CGTase) gene derived from a microorganism of genus Bacillus and the amino acid sequence of CGTase are determined. A recombinant DNA carrying the CGTase gene is introduced by in vitro genetic engineering technique into a host microorganism of species *Bacillus subtilis* or *Escherichia coli.* The recombinant microorganism carrying the recombinant DNA autonomically proliferates to secrete a large amount of CGTase.

25 Claims, 16 Drawing Sheets

FIG. 1

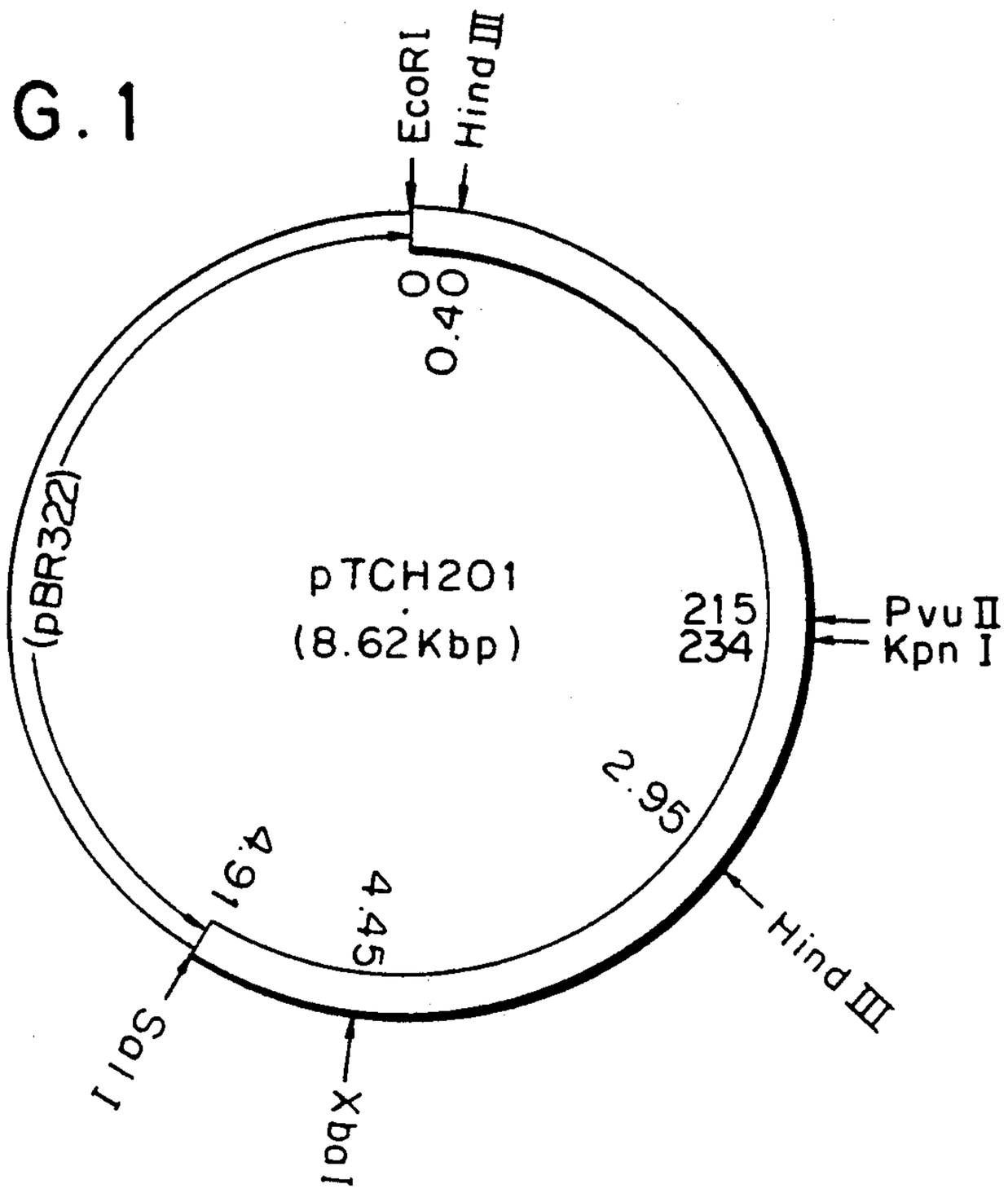
EcoRI
Hind III
0
0.40
pTCH201
(8.62Kbp)
215
234
Pvu II
Kpn I
2.95
Hind III
4.45
Xba I
4.91
Sal I
(pBR322)

FIG. 2

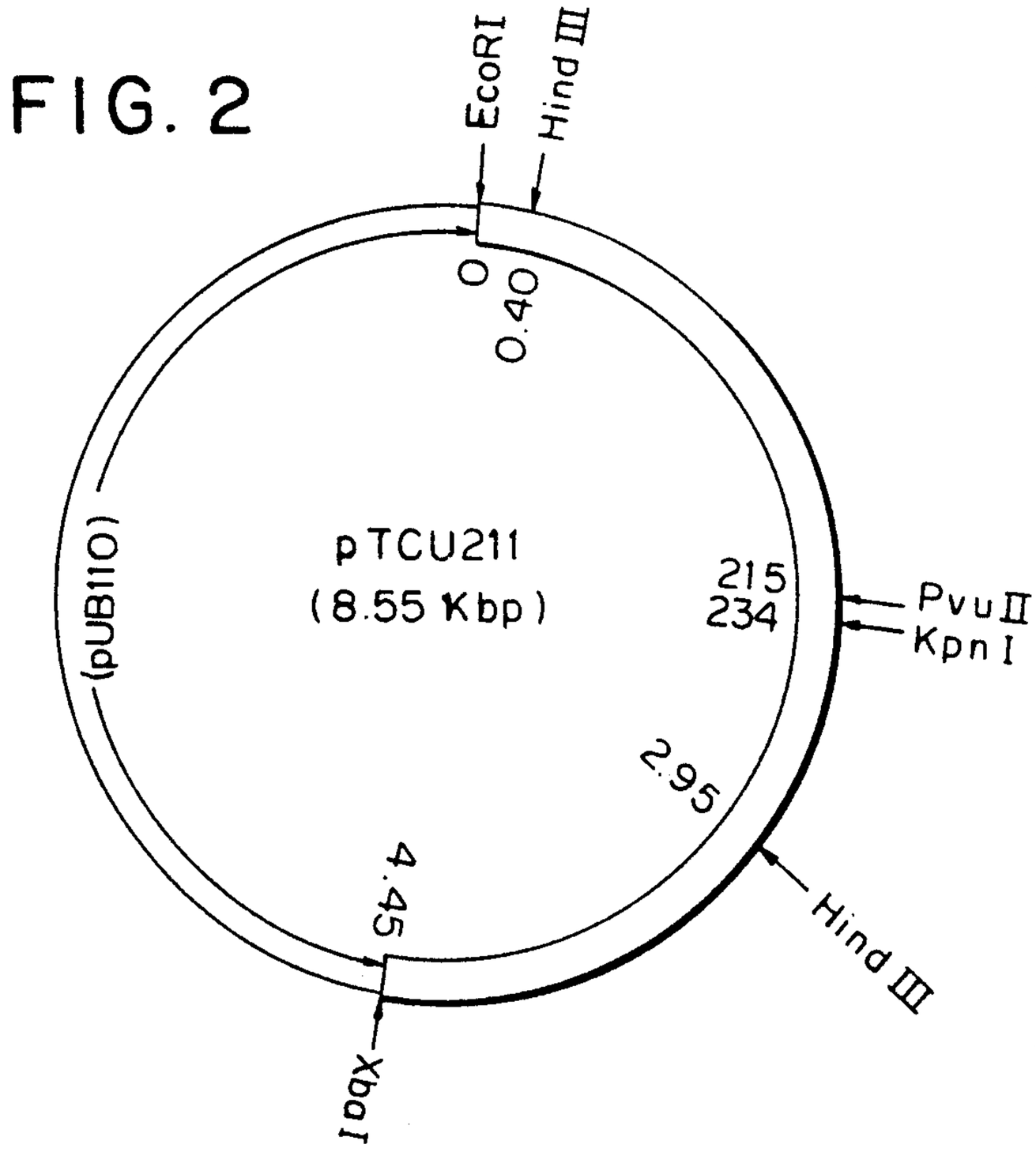
EcoRI
Hind III
0
0.40
pTCU211
(8.55 Kbp)
215
234
Pvu II
Kpn I
2.95
Hind III
4.45
Xba I
(pUB110)

FIG. 3

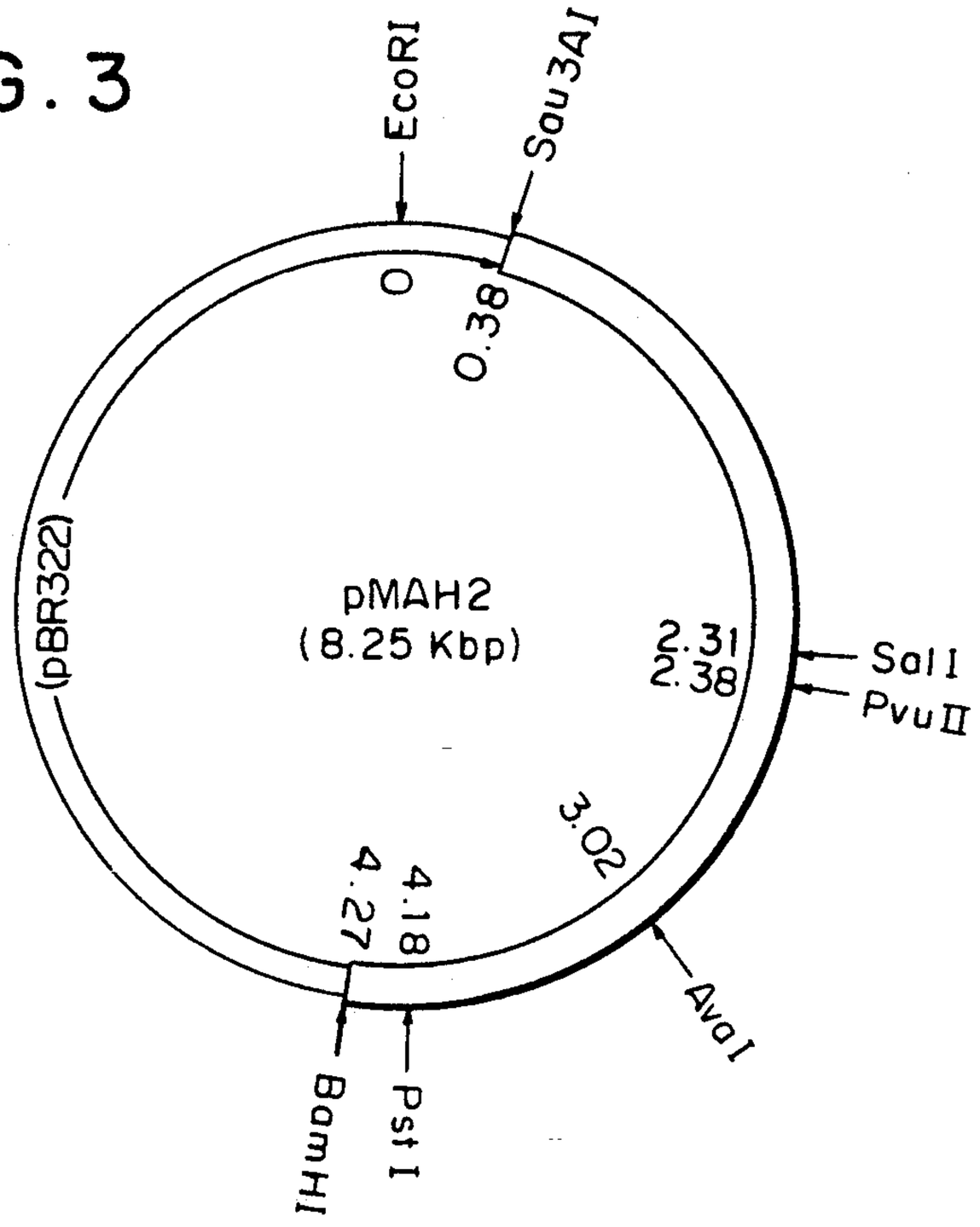
EcoRI
Sau3AI
0
0.38
pMAH2
(8.25 Kbp)
2.31
2.38
SalI
PvuII
3.02
4.18
4.27
AvaI
BamHI
PstI
(pBR322)

FIG. 4

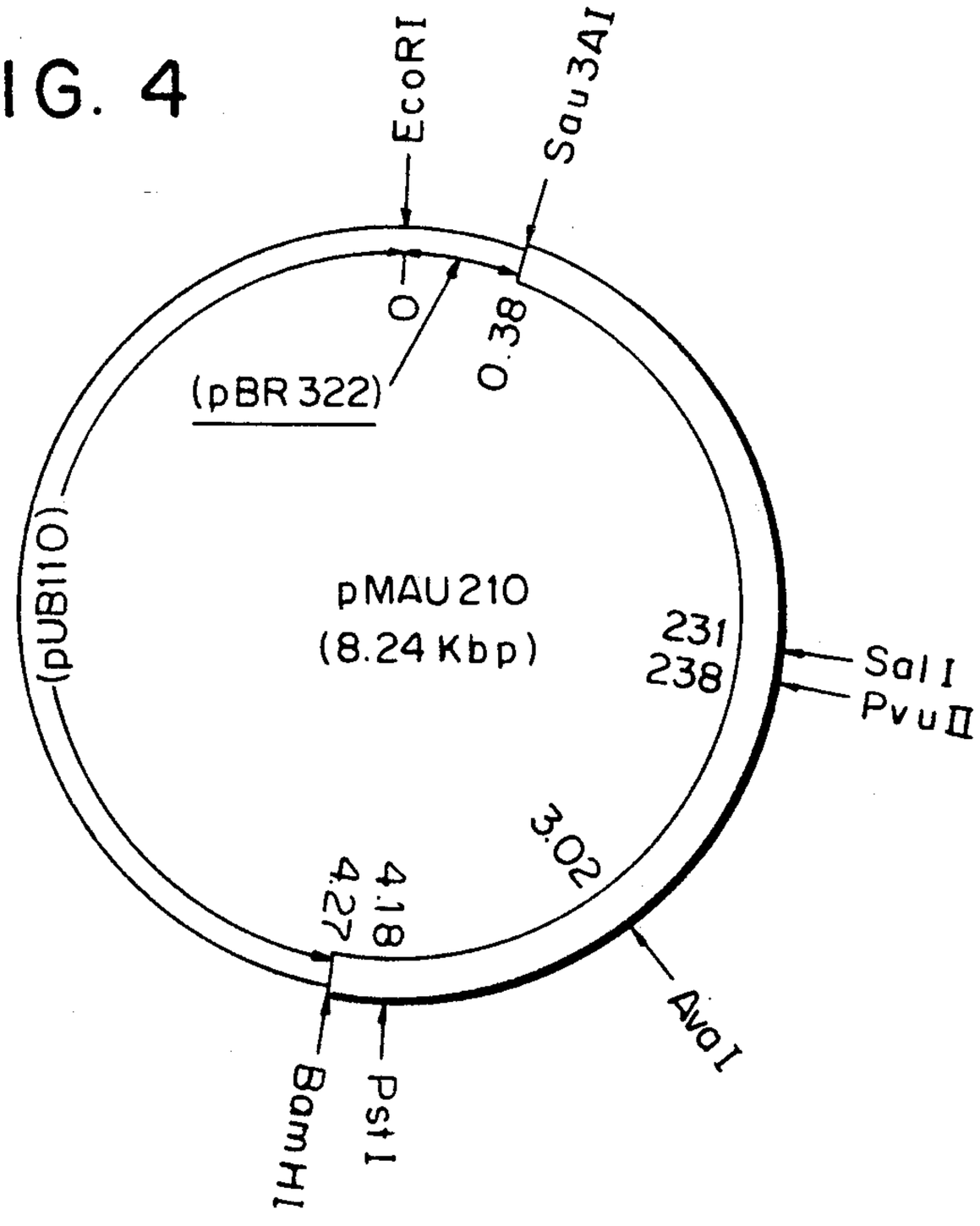
EcoRI
Sau3AI
0
0.38
(pBR322)
pMAU210
(8.24 Kbp)
231
238
SalI
PvuII
3.02
4.18
4.27
AvaI
BamHI
PstI
(pUB110)

FIG. 5A

10	20	30	40	50	60
GCTGGAAATC	TTAA TAAGGT	AAACT TTACA	TCAGATG TTG	TCTATCAAAT	TGTAGTGGAT
70	80	90	100	110	120
CGAT TTGTGG	ATGGAAATAC	ATCCAA TAAT	CCGAGTGGAG	CATTATTTAG	CTCAGGATGT
130	140	150	160	170	180
ACGA ATTTAC	GCAAGTATTG	CGGTGGAGAT	TGGCAAGGCA	TA TCAAT AA	AAT TAACGAT
190	200	210	220	230	240
GGGTAT TTAA	CAGATATGGG	TGTGACAGCG	ATAT GGATTT	CTCAGCCTGT	AGAAAATGTA
250	260	270	280	290	300
TT TTCTGTGA	TGAATGATGC	AAGCGGTTCC	GCATCCTATC	ATGG TTATTG	GGCGCGCGAT
310	320	330	340	350	360
TTCAAAAAGC	CAAACCCGTT	TT TTGGTACC	CTCAGTGAT T	TCCAACGTTT	AGTTGATGCC
370	380	390	400	410	420
GCACATGCAA	AAGGAATAAA	GGTAATTATT	GAC TTTGCCC	CCAACCATAC	TTCTCCTGCT
430	440	450	460	470	480
TCAGAAACGA	AT CCT TCTTA	TATGGAAAAC	GGACGACTGT	ACGATAATGG	GACATTGCTT
490	500	510	520	530	540
GGCGGTTACA	CAAATGATGC	CAACATGTAT	T TTCACCA TA	ACGGTGGAAC	AACGTTTTCC

FIG. 5B

550	560	570	580	590	600
AGCTT AGAGG	ATGGGATTTA	TCGAAATCTG	TTTGACT TGG	CGGACCTTAA	CCATCAGAAC
610	620	630	640	650	660
CCTGT TA TTG	ATAGG TATTT	AAAAGATGCA	GTAAAAA TGT	GGA TAGATAT	GGGGATTGAT
670	680	690	700	710	720
GGTAT CCGTA	TGGATGCGGT	GAAGCACATG	CCGTTTG GAT	GGCAAAAATC	TCTGATGGAT
730	740	750	760	770	780
GAGAT TGATA	AC TATCGTCC	TGTCTTTACG	TT TGGGGAGT	GG TTTTTGTC	AGAAAATGAA
790	800	810	820	830	840
GTGGACGCGA	ACAATCAT TA	CTTTGCCAAT	GAAAGTGG AA	TGAGT TTGCT	CGAT TTTCGT
850	860	870	880	890	900
TTCGGACAAA	AGCTTCGTCA	AGTATTGCGC	AATAACAGCG	ATAAT TGGTA	TGGC TTTAAT
910	920	930	940	950	960
CAAATGATTC	AAGATACGGC	ATCAGCATAT	GACGAGGTTC	TCG ATCAAGT	AACAT TCATA
970	980	990	1000	1010	1020
GACAACCATG	AT ATGGATCG	GTT TATGATT	GACGGAGGAG	ATCCGCGCAA	GGTGGATATG
1030	1040	1050	1060	1070	1080
GCACTTGCTG	TA TTATTGAC	ATCCCGTGGC	GTACCGAA TA	T TTACTATGG	TACAGAGCAA

FIG. 5C

1090	1100	1110	1120	1130	1140
TACATGACCG	GTAACGGCGA	TCCAAACAAT	CGTAAGATGA	TGAGTTCATT	CAA TAAAAAT
1150	1160	1170	1180	1190	1200
ACTCGCGCGT	ATCAAGT GAT	TCAAAAACTA	TCT TCTCTCC	GACGAAACAA	TCCGGCGTTA
1210	1220	1230	1240	1250	1260
GCT TATGGTG	ATACGGAACA	GCGTTGGATC	AATGGCGATG	TG TATGTGT A	TGAGCGACAG
1270	1280	1290	1300	1310	1320
T TTGGCAAAG	ATG TTGTGTT	AGTT CGGGTT	AATCGT AGTT	CAAGCAGTAA	TTAC TCGATT
1330	1340	1350	1360	1370	1380
ACTGGC TTAT	TTACAGCTTT	ACCAGCAGGA	ACATATACGG	ATCAGCT TGG	CGGTC TTTTA
1390	1400	1410	1420	1430	1440
GACGGAAATA	CAA TTCAAGT	CGGTTCAAAT	GGATCAGT TA	ATGCATT TGA	CTTAGGACCG
1450	1460	1470	1480	1490	1500
GGGGAAGTCG	GTGTATGGGC	ATACAGTGCA	ACAGAAAGCA	CGCCAATTAT	TGGTCATGT T
1510	1520	1530	1540	1550	1560
GGACCGATGA	TGGGGCAAGT	CGGTCATCAA	GTAACCATTG	ATGGCGAAGG	ATTCGGAACA
1570	1580	1590	1600	1610	1620
AATACGGGCA	CTGTGAAGTT	CGGAACGACA	GCTGCCAATG	TTGTG TCT TG	GTCTAACAAT

FIG. 5D

1630	1640	1650	1660	1670	1680
CAAATCGTTG	TGGCTGTACC	AAATGTG TCA	CCAGGAAAAT	ATAATATTAC	CGTCCAATCA
1690	1700	1710	1720	1730	1740
TCAAGCGGTC	AAACGAGTGC	GGCTTATGAT	AAC TTTGAAG	TACTAACAAA	TGATCAAGTG
1750	1760	1770	1780	1790	1800
TCAGTGCGGT	TTGT TGTTAA	TAACGCGACT	ACCAATCTAG	GGCAAAA TAT	ATACATTG TT
1810	1820	1830	1840	1850	1860
GGCAACGTAT	ATGAGCTCGG	CAACTGGGAC	ACTAGTAAGG	CAATCGGTCC	AATGT TCAAT
1870	1880	1890	1900	1910	1920
CAAGTGGT TT	ACTCCTATCC	TACATGGTA T	ATAGATGTCA	GTGTCCCAGA	AGGAAAGACA
1930	1940	1950	1960	1970	1980
ATT GAGTT TA	AGT TTATTAA	AAAAGACAGC	CAAGGTAATG	TCACTTGGGA	AAGTGGTTCA
1990	2000	2010	2020	2030	2040
AATCATG T TT	ATACGACACC	AACGAATACA	ACCGGAAAAA	TTATAGTGGA	TTGGCAGAAC

FIG. 6

```
                10                  20                  30                  40                  50                  60
ATGAGAAGAT  GGC TTTCGCT  AGTC TTGAGC  ATGTCATTTG  TATTTAGTGC  AATTTTTATA

                70                  80                  90                 100
GTATCT GATA  CGCAGAAAGT  CACCGTTG AA  GCA
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FIG. 7

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1>	Met	Arg	Arg	Trp	Leu	Ser	Leu	Val	Leu	Ser	Met	Ser	Phe	Val	Phe
16>	Ser	Ala	Ile	Phe	Ile	Val	Ser	Asp	Thr	Gln	Lys	Val	Thr	Val	Glu
31>	Ala														

FIG. 8A

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1>	Ala	Gly	Asn	Leu	Asn	Lys	Val	Asn	Phe	Thr	Ser	Asp	Val	Val	Tyr
16>	Gln	Ile	Val	Val	Asp	Arg	Phe	Val	Asp	Gly	Asn	Thr	Ser	Asn	Asn
31>	Pro	Ser	Gly	Ala	Leu	Phe	Ser	Ser	Gly	Cys	Thr	Asn	Leu	Arg	Lys
46>	Tyr	Cys	Gly	Gly	Asp	Trp	Gln	Gly	Ile	Ile	Asn	Lys	Ile	Asn	Asp
61>	Gly	Tyr	Leu	Thr	Asp	Met	Gly	Val	Thr	Ala	Ile	Trp	Ile	Ser	Gln
76>	Pro	Val	Glu	Asn	Val	Phe	Ser	Val	Met	Asn	Asp	Ala	Ser	Gly	Ser
91>	Ala	Ser	Tyr	His	Gly	Tyr	Trp	Ala	Arg	Asp	Phe	Lys	Lys	Pro	Asn
106>	Pro	Phe	Phe	Gly	Thr	Leu	Ser	Asp	Phe	Gln	Arg	Leu	Val	Asp	Ala
121>	Ala	His	Ala	Lys	Gly	Ile	Lys	Val	Ile	Ile	Asp	Phe	Ala	Pro	Asn
136>	His	Thr	Ser	Pro	Ala	Ser	Glu	Thr	Asn	Pro	Ser	Tyr	Met	Glu	Asn
151>	Gly	Arg	Leu	Tyr	Asp	Asn	Gly	Thr	Leu	Leu	Gly	Gly	Tyr	Thr	Asn
166>	Asp	Ala	Asn	Met	Tyr	Phe	His	His	Asn	Gly	Gly	Thr	Thr	Phe	Ser
181>	Ser	Leu	Glu	Asp	Gly	Ile	Tyr	Arg	Asn	Leu	Phe	Asp	Leu	Ala	Asp
196>	Leu	Asn	His	Gln	Asn	Pro	Val	Ile	Asp	Arg	Tyr	Leu	Lys	Asp	Ala
211>	Val	Lys	Met	Trp	Ile	Asp	Met	Gly	Ile	Asp	Gly	Ile	Arg	Met	Asp
226>	Ala	Val	Lys	His	Met	Pro	Phe	Gly	Trp	Gln	Lys	Ser	Leu	Met	Asp
241>	Glu	Ile	Asp	Asn	Tyr	Arg	Pro	Val	Phe	Thr	Phe	Gly	Glu	Trp	Phe
256>	Leu	Ser	Glu	Asn	Glu	Val	Asp	Ala	Asn	Asn	His	Tyr	Phe	Ala	Asn
271>	Glu	Ser	Gly	Met	Ser	Leu	Leu	Asp	Phe	Arg	Phe	Gly	Gln	Lys	Leu
286>	Arg	Gln	Val	Leu	Arg	Asn	Asn	Ser	Asp	Asn	Trp	Tyr	Gly	Phe	Asn
301>	Gln	Met	Ile	Gln	Asp	Thr	Ala	Ser	Ala	Tyr	Asp	Glu	Val	Leu	Asp
316>	Gln	Val	Thr	Phe	Ile	Asp	Asn	His	Asp	Met	Asp	Arg	Phe	Met	Ile
331>	Asp	Gly	Gly	Asp	Pro	Arg	Lys	Val	Asp	Met	Ala	Leu	Ala	Val	Leu
346>	Leu	Thr	Ser	Arg	Gly	Val	Pro	Asn	Ile	Tyr	Tyr	Gly	Thr	Glu	Gln
361>	Tyr	Met	Thr	Gly	Asn	Gly	Asp	Pro	Asn	Asn	Arg	Lys	Met	Met	Ser
376>	Ser	Phe	Asn	Lys	Asn	Thr	Arg	Ala	Tyr	Gln	Val	Ile	Gln	Lys	Leu

FIG. 8B

391>	Ser	Ser	Leu	Arg	Arg	Asn	Asn	Pro	Ala	Leu	Ala	Tyr	Gly	Asp	Thr
406>	Glu	Gln	Arg	Trp	Ile	Asn	Gly	Asp	Val	Tyr	Val	Tyr	Glu	Arg	Gln
421>	Phe	Gly	Lys	Asp	Val	Val	Leu	Val	Arg	Val	Asn	Arg	Ser	Ser	Ser
436>	Ser	Asn	Tyr	Ser	Ile	Thr	Gly	Leu	Phe	Thr	Ala	Leu	Pro	Ala	Gly
451>	Thr	Tyr	Thr	Asp	Gln	Leu	Gly	Gly	Leu	Leu	Asp	Gly	Asn	Thr	Ile
466>	Gln	Val	Gly	Ser	Asn	Gly	Ser	Val	Asn	Ala	Phe	Asp	Leu	Gly	Pro
481>	Gly	Glu	Val	Gly	Val	Trp	Ala	Tyr	Ser	Ala	Thr	Glu	Ser	Thr	Pro
496>	Ile	Ile	Gly	His	Val	Gly	Pro	Met	Met	Gly	Gln	Val	Gly	His	Gln
511>	Val	Thr	Ile	Asp	Gly	Glu	Gly	Phe	Gly	Thr	Asn	Thr	Gly	Thr	Val
526>	Lys	Phe	Gly	Thr	Thr	Ala	Ala	Asn	Val	Val	Ser	Trp	Ser	Asn	Asn
541>	Gln	Ile	Val	Val	Ala	Val	Pro	Asn	Val	Ser	Pro	Gly	Lys	Tyr	Asn
556>	Ile	Thr	Val	Gln	Ser	Ser	Ser	Gly	Gln	Thr	Ser	Ala	Ala	Tyr	Asp
571>	Asn	Phe	Glu	Val	Leu	Thr	Asn	Asp	Gln	Val	Ser	Val	Arg	Phe	Val
586>	Val	Asn	Asn	Ala	Thr	Thr	Asn	Leu	Gly	Gln	Asn	Ile	Tyr	Ile	Val
601>	Gly	Asn	Val	Tyr	Glu	Leu	Gly	Asn	Trp	Asp	Thr	Ser	Lys	Ala	Ile
616>	Gly	Pro	Met	Phe	Asn	Gln	Val	Val	Tyr	Ser	Tyr	Pro	Thr	Trp	Tyr
631>	Ile	Asp	Val	Ser	Val	Pro	Glu	Gly	Lys	Thr	Ile	Glu	Phe	Lys	Phe
646>	Ile	Lys	Lys	Asp	Ser	Gln	Gly	Asn	Val	Thr	Trp	Glu	Ser	Gly	Ser
661>	Asn	His	Val	Tyr	Thr	Thr	Pro	Thr	Asn	Thr	Thr	Gly	Lys	Ile	Ile
676>	Val	Asp	Trp	Gln	Asn										

FIG. 9A

10	20	30	40	50	60
TCCCCGGATA	CGAGCGTGAA	CAACAAGCTC	AAT TTTAGCA	CGGA TACGGT	T TACCAGATT
70	80	90	100	110	120
GTAACCGACC	GGTTTGTGGA	CGGCAAT TCC	GCCAACAACC	CGACCGGAGC	AGCCTTCAGC
130	140	150	160	170	180
AGCGATCA TT	CCAACCTGAA	GCTGTATTTC	GGGGGCGACT	GGCAGGGGAT	CACGAACAAA
190	200	210	220	230	240
ATCAACGACG	GCTATCTGAC	CGGAATGGGC	ATCACCGCCC	TCTGGA TCTC	GCAGCCGGTT
250	260	270	280	290	300
GAGAACATCA	CCGCCGTCAT	CAATTA TTCG	GGCGTCAACA	ATACAG CTTA	CC ACGGTTAC
310	320	330	340	350	360
TGGCCTCGCG	ACTTCAAGAA	GACCAATGCC	GCGTTCGGCA	GCTTCACCGA	CT TC TCCAAT
370	380	390	400	410	420
TTGATCGCCG	CAGCGCATTC	ACACAATATC	AAGGTAGT TA	TGGACTT TGC	ACCT AATCAC
430	440	450	460	470	480
ACCAACCCGG	CTTCGAGTAC	GGACCCCTCG	TTCGCCGAGA	ACGGCGCGCT	CTACAACAAC
490	500	510	520	530	540
GGAACGCTGC	TCGGCAAGTA	TAGCAACGAT	ACCGCCGGCC	TG TTCCACCA	CAATGGCGGC

FIG. 9B

550	560	570	580	590	600
ACCGAT TTC T	CGACGACTGA	AAGCGGTATC	TACAAGAACC	TGTACGATCT	CGCGGATATC
610	620	630	640	650	660
AATCAGA ACA	ACAACACCAT	CGACTCGTAT	CTCAAGGAAT	CGATCCAGCT	GTGGCTGAAT
670	680	690	700	710	720
CTCGGAGTCG	ACGGCATCCG	CTTCGACGCC	GTGAAGCATA	TGCCTCAGGG	CTGGCAGAAG
730	740	750	760	770	780
AGCTACG TCT	CGTCGATCTA	CAGCAGCGCC	AATCCGGTGT	TCACC TTCGG	TGAATGGTTC
790	800	810	820	830	840
CTCGGCCCCG	ACGAAATGAC	CCAGGACAAC	ATCAACTTCG	CGAATCAGAG	CGGCATGCAC
850	860	870	880	890	900
CTGCTG GACT	TTGCGTTTGC	GCAGGAAATC	CGTGAAGTGT	TCCGCGACAA	GTCGGAGACG
910	920	930	940	950	960
ATGACCGACC	TGAACTCGGT	GATCTCCAGC	ACCGGCTCCA	GCTATAA TTA	CATCAACAAC
970	980	990	1000	1010	1020
ATGG TTACGT	TCATCGACAA	CCATGACATG	GACCGCTTCC	AGCAAGCCGG	AGCGAGCACT
1030	1040	1050	1060	1070	1080
CGCCCGACCG	AGCAGGCTCT	TGCGGTAACG	CTGACTTCCC	GCGGCGTTCC	GGCAATCTAC

FIG. 9C

1090	1100	1110	1120	1130	1140
TACGGTACAG	AGCAA TATAT	GACCGGCAAC	GGCGACCCGA	ACAACCGCGG	CATGATGACC
1150	1160	1170	1180	1190	1200
GGCTTCGATA	CGAACAAGAC	AGCGTACAAA	GT GATCAAGG	CGCTGGCTCC	GCTTCGCAAG
1210	1220	1230	1240	1250	1260
TCCAACCCGG	CTCTCGCCTA	CGGCTCGACG	AC CCAGCGTT	GGGTGAACAG	CGACGTCTAC
1270	1280	1290	1300	1310	1320
GTAT ATGAAC	GCAAGTTCGG	AAGCAACGTA	GC T TTCGTTG	CCGTCAACCG	CAGCTCGACG
1330	1340	1350	1360	1370	1380
ACTGCCT ATC	CGATATCGGG	AGCGC TTACT	GC TCTGCCAA	ACGGAACGTA	TACCGACGTT
1390	1400	1410	1420	1430	1440
CTCGGCGGCC	TGC TTAATGG	CAATT CAATT	AC CGTTAACG	GCGGCACGGT	CAGCAA CTTT
1450	1460	1470	1480	1490	1500
ACACT TGCAG	CGGGCGGTAC	GGCAGTCTGG	CAGTACACGA	CGACGGAATC	CTCGCC GATT
1510	1520	1530	1540	1550	1560
ATCGGCAACG	TCGGCCCGAC	TATGGGCAAG	CCCGGCAACA	CCATCACGAT	CGACGGACGC
1570	1580	1590	1600	1610	1620
GGCTT CGGTA	CTACGAAGAA	CAAAGTT ACT	T TCGGTACGA	CAGCCGTTAC	CGGCGCGAAC

FIG. 9D

1630	1640	1650	1660	1670	1680
ATCGTGAGCT	GGGAAGATAC	CGAAATCAAG	GTCAAAG TTC	CGAACGTGGC	CGCCGGCAAC
1690	1700	1710	1720	1730	1740
ACGGCCGTTA	CGGTAACGAA	CGCCGCCGGC	ACTACCAGCG	CAGCGTTCAA	CAACT T TAAC
1750	1760	1770	1780	1790	1800
GTACTGACTG	CCGA TCAGGT	CACTGTCCGC	TTCAAAGTCA	ACAATGCCAC	CACGGCCCTG
1810	1820	1830	1840	1850	1860
GGACAAAACG	TCTACCTGAC	CGGTAACGTC	GCCGAGCTTG	GCAACTGGAC	AGCCGCCAAC
1870	1880	1890	1900	1910	1920
GCAATCGGTC	CGATGTACAA	CCAGGTAGAA	GCCAGCTATC	CGAC TTGGTA	CT TCGACGTC
1930	1940	1950	1960	1970	1980
AGCGTTCCGG	CCAACACGGC	GCTGCAATTC	AA GTTCATCA	AAGTGAACGG	CT CGACAGTG
1990	2000	2010	2020	2030	2040
ACTTGGGAAG	GCGGCAACAA	CCACACCTTC	ACCTCGCCTT	CGAGCGGCGT	TGCGACCGTA
2050	2060				
ACGGTCGATT	GGCAGAAC				

FIG. 10

```
                10                  20                  30                  40                  50                  60
A TGAAAAAGC  AAGTCAAATG  GTTGACGTCG  GTGTCGATGT  CCGTAGGGAT  CGCACTCGGC

                70                  80                  90
GCGGCGCTGC  CTGTATGGGC  A
```

FIG. 11

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1>	Met	Lys	Lys	Gln	Val	Lys	Trp	Leu	Thr	Ser	Val	Ser	Met	Ser	Val
16>	Gly	Ile	Ala	Leu	Gly	Ala	Ala	Leu	Pro	Val	Trp	Ala			

FIG. 12A

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1>	Ser	Pro	Asp	Thr	Ser	Val	Asn	Asn	Lys	Leu	Asn	Phe	Ser	Thr	Asp
16>	Thr	Val	Tyr	Gln	Ile	Val	Thr	Asp	Arg	Phe	Val	Asp	Gly	Asn	Ser
31>	Ala	Asn	Asn	Pro	Thr	Gly	Ala	Ala	Phe	Ser	Ser	Asp	His	Ser	Asn
46>	Leu	Lys	Leu	Tyr	Phe	Gly	Gly	Asp	Trp	Gln	Gly	Ile	Thr	Asn	Lys
61>	Ile	Asn	Asp	Gly	Tyr	Leu	Thr	Gly	Met	Gly	Ile	Thr	Ala	Leu	Trp
76>	Ile	Ser	Gln	Pro	Val	Glu	Asn	Ile	Thr	Ala	Val	Ile	Asn	Tyr	Ser
91>	Gly	Val	Asn	Asn	Thr	Ala	Tyr	His	Gly	Tyr	Trp	Pro	Arg	Asp	Phe
106>	Lys	Lys	Thr	Asn	Ala	Ala	Phe	Gly	Ser	Phe	Thr	Asp	Phe	Ser	Asn
121>	Leu	Ile	Ala	Ala	Ala	His	Ser	His	Asn	Ile	Lys	Val	Val	Met	Asp
136>	Phe	Ala	Pro	Asn	His	Thr	Asn	Pro	Ala	Ser	Ser	Thr	Asp	Pro	Ser
151>	Phe	Ala	Glu	Asn	Gly	Ala	Leu	Tyr	Asn	Asn	Gly	Thr	Leu	Leu	Gly
166>	Lys	Tyr	Ser	Asn	Asp	Thr	Ala	Gly	Leu	Phe	His	His	Asn	Gly	Gly
181>	Thr	Asp	Phe	Ser	Thr	Thr	Glu	Ser	Gly	Ile	Tyr	Lys	Asn	Leu	Tyr
196>	Asp	Leu	Ala	Asp	Ile	Asn	Gln	Asn	Asn	Asn	Thr	Ile	Asp	Ser	Tyr
211>	Leu	Lys	Glu	Ser	Ile	Gln	Leu	Trp	Leu	Asn	Leu	Gly	Val	Asp	Gly
226>	Ile	Arg	Phe	Asp	Ala	Val	Lys	His	Met	Pro	Gln	Gly	Trp	Gln	Lys
241>	Ser	Tyr	Val	Ser	Ser	Ile	Tyr	Ser	Ser	Ala	Asn	Pro	Val	Phe	Thr
256>	Phe	Gly	Glu	Trp	Phe	Leu	Gly	Pro	Asp	Glu	Met	Thr	Gln	Asp	Asn
271>	Ile	Asn	Phe	Ala	Asn	Gln	Ser	Gly	Met	His	Leu	Leu	Asp	Phe	Ala
286>	Phe	Ala	Gln	Glu	Ile	Arg	Glu	Val	Phe	Arg	Asp	Lys	Ser	Glu	Thr
301>	Met	Thr	Asp	Leu	Asn	Ser	Val	Ile	Ser	Ser	Thr	Gly	Ser	Ser	Tyr
316>	Asn	Tyr	Ile	Asn	Asn	Met	Val	Thr	Phe	Ile	Asp	Asn	His	Asp	Met
331>	Asp	Arg	Phe	Gln	Gln	Ala	Gly	Ala	Ser	Thr	Arg	Pro	Thr	Glu	Gln
346>	Ala	Leu	Ala	Val	Thr	Leu	Thr	Ser	Arg	Gly	Val	Pro	Ala	Ile	Tyr
361>	Tyr	Gly	Thr	Glu	Gln	Tyr	Met	Thr	Gly	Asn	Gly	Asp	Pro	Asn	Asn
376>	Arg	Gly	Met	Met	Thr	Gly	Phe	Asp	Thr	Asn	Lys	Thr	Ala	Tyr	Lys

FIG. 12B

391> Val Ile Lys Ala Leu Ala Pro Leu Arg Lys Ser Asn Pro Ala Leu
406> Ala Tyr Gly Ser Thr Thr Gln Arg Trp Val Asn Ser Asp Val Tyr
421> Val Tyr Glu Arg Lys Phe Gly Ser Asn Val Ala Leu Val Ala Val
436> Asn Arg Ser Ser Thr Thr Ala Tyr Pro Ile Ser Gly Ala Leu Thr
451> Ala Leu Pro Asn Gly Thr Tyr Thr Asp Val Leu Gly Gly Leu Leu
466> Asn Gly Asn Ser Ile Thr Val Asn Gly Gly Thr Val Ser Asn Phe
481> Thr Leu Ala Ala Gly Gly Thr Ala Val Trp Gln Tyr Thr Thr Thr
496> Glu Ser Ser Pro Ile Ile Gly Asn Val Gly Pro Thr Met Gly Lys
511> Pro Gly Asn Thr Ile Thr Ile Asp Gly Arg Gly Phe Gly Thr Thr
526> Lys Asn Lys Val Thr Phe Gly Thr Thr Ala Val Thr Gly Ala Asn
541> Ile Val Ser Trp Glu Asp Thr Glu Ile Lys Val Lys Val Pro Asn
556> Val Ala Ala Gly Asn Thr Ala Val Thr Val Thr Asn Ala Ala Gly
571> Thr Thr Ser Ala Ala Phe Asn Asn Phe Asn Val Leu Thr Ala Asp
586> Gln Val Thr Val Arg Phe Lys Val Asn Asn Ala Thr Thr Ala Leu
601> Gly Gln Asn Val Tyr Leu Thr Gly Asn Val Ala Glu Leu Gly Asn
616> Trp Thr Ala Ala Asn Ala Ile Gly Pro Met Tyr Asn Gln Val Glu
631> Ala Ser Tyr Pro Thr Trp Tyr Phe Asp Val Ser Val Pro Ala Asn
646> Thr Ala Leu Gln Phe Lys Phe Ile Lys Val Asn Gly Ser Thr Val
661> Thr Trp Glu Gly Gly Asn Asn His Thr Phe Thr Ser Pro Ser Ser
676> Gly Val Ala Thr Val Thr Val Asp Trp Gln Asn

POLYPEPTIDE POSSESSING CYCLOMALTODEXTRIN GLUCANOTRANSFERASE ACTIVITY

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a continuation of application Ser. No. 07/438,933, filed Nov. 22, 1989, now abandoned which was a continuation of application Ser. No. 06/804,487, filed Dec. 4, 1985, now abandoned.

FIELD OF THE INVENTION

The present invention relates to a polypeptide, and particularly a polypeptide possessing cyclomaltodextrin glucanotransferase activity. The present invention also relates to DNA, microorganisms and processes related to the production of such polypeptide.

ABBREVIATIONS

Throughout the present specification and claims, amino acids, peptides, etc., are designated with abbreviations which are commonly used in the art. Examples of such abbreviations are as follows.

When optical isomers are possible, the abbreviations of amino acids mean L-isomers, unless specified otherwise.

DNA is the abbreviation of deoxyribonucleic acid; RNA ribonucleic acid; A, adenine; T, thymine; G, guanine; C, cytosine; dNTP, deoxynucleotide triphosphate; ddNTP, dideoxynucleotide triphosphate; dCTP, deoxycytidin triphosphate; SDS, sodium dodecyl sulfate; Ala, alanine; Arg, arginine; Asn, asparagine; Asp, aspartic acid; Cys, cysteine; Gln, glutamine; Glu, glutamic acid; Gly, glycine; His, histidine; Ile, isoleucine; Leu, leucine; Lys, lysine; Met, methionine; Phe, phenylalanine; Pro, proline; Ser, serine; Thr, threonine; Trp, tryptophan; Tyr, tyrosine; Val, valine; and CGTase, cyclomaltodextrin glucanotransferase.

The wording of "polypeptide" means "polypeptide possessing CGTase activity".

DESCRIPTION OF THE PRIOR ART

CGTase, or macerans, has been known for years as an enzyme produced by *Bacillus macerans.*

Recently, it was found that CGTase is produced by other microorganisms such as those of species *Bacillus stearothermophilus* and *Bacillus circulans.* The saccharide transfer activity of CGTase now has many industrial uses.

For example, cyclodextrins are produced by subjecting gelatinized starch to the action of CGTase, while glycosylsucrose production utilizes the saccharide transfer reaction from starch to sucrose which is effected by subjecting a mixture solution of liquefied starch and sucrose to CGTase.

Cyclodextrins are now expanding as a host for forming stable inclusion complexes with organic compounds which are volatile or susceptible to oxidation. Demand for glycosylsucrose is also expanding as a mildly-sweet low-cariogenic sweetener which is commercialized by Hayashibara Co., Ltd., Okayama, Japan, under the Registered Trademark of "Coupling Sugar".

In order to meet these demands, development of means to provide a constant CGTase supply is an urgent necessity. This requires determination of the amino acid sequence of the polypeptide that possesses CGTase activity.

Such amino acid sequence has , however, so far been unknown.

BRIEF DESCRIPTION OF THE ACCOMPANYING DRAWINGS

FIG. **1** shows the restriction map of recombinant DNA pTCH201, in particular that of the DNA fragment which carries the polypeptide gene derived from *Bacillus stearothermophilus.*

FIG. **2** shows the restriction map of recombinant DNA pTCU211, in particular that of the DNA fragment which carries the polypeptide gene derived from *Bacillus stearothermophilus.*

FIG. **3** shows the restriction map of recombinant DNA pMAH2, in particular that of the DNA fragment which carries the polypeptide gene derived from *Bacillus macerans.*

FIG. **4** shows the restriction map of recombinant DNA pMAU210, in particular that of the DNA fragment which carries the polypeptide gene derived from *Bacillus macerans.*

FIGS. **5**(A–D) show the nucleotide sequence of the polypeptide gene derived from *Bacillus stearothermophilus.*

FIG. **6** shows the nucleotide sequence of the signal peptide gene located upstream of the 5'-terminal end of the polypeptide gene of FIG. **5**.

FIG. **7** shows the amino acid sequence of the signal peptide of FIG. **6**.

FIGS. **8**(A–B) show the amino acid sequence of the polypeptide determined with reference to the sequence shown in FIG. **5**.

FIGS. **9**(A–D) show the nucleotide sequence of the polypeptide gene derived from *Bacillus macerans.*

FIG. **10** shows the sequence of the signal peptide located upstream of the 5'-site of the polypeptide of FIG. **9**.

FIG. **11** shows the amino acid sequence of the signal peptide of FIGS. **10**(A–B).

FIGS. **12**(A–B) show the amino acid sequence of the polypeptide derived from *Bacillus macerans.*

SUMMARY OF THE INVENTION

The present inventors carried out investigations to determine the amino acid sequence of CGTase polypeptide; to assure a wide polypeptide availability by recombinant gene technology; and also to improve polypeptide productivity.

As a result, the present inventors found that the CGTase polypeptide comprises one or more partial amino acid sequences selected from the group consisting of

(a) Asn-Lys-Ile-Asn-Asp-Gly-Tyr-Leu-Thr,

(b) Pro-Val-Phe-Thr-Phe-Gly-Glu-Trp-Phe-Leu,

(c) Val-Thr-Phe-Ile-Asp-Asn-His-Asp-Met-Asp-Arg-Phe,

(d) Ile-Tyr-Tyr-Gly-Thr-Glu-Gln-Tyr-Met-Thr-Gly-Asn-Gly-Asp-Pro-Asn-Asn-Arg, and

(e) Asn-Pro-Ala-Leu-Ala-Tyr-Gly,

and that, more particularly, these partial amino acids sequences (a), (b), (c), (d) and (e) are located in order of nearness to the N-terminal end of the polypeptide.

The polypeptide is characterized by the facts that it forms cyclodextrin from soluble starch; that it shows a molecular weight of 70,000±10,000 daltons on SDS-

polyacrylamide electrophoresis; and that it has a specific activity of 200±30 units/mg protein.

The present inventors also found that polypeptides derived from *Bacillus stearothermophilus* and *Bacillus macerans* have the amino acid sequences as shown in FIGS. **8** and **12**, respectively. Both amino acid sequences will be discussed hereafter.

In addition, the present inventors determined the amino acid sequences of the signal peptides which regulate polypeptide secretion from producer microorganisms.

The present invention and features thereof will hereinafter be explained.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

In the present invention, the amino acid sequence of the CGTase polypeptide is determined by cloning the polypeptide gene from a CGTase producer microorganism and sequencing the polypeptide gene.

The amino acid sequence containing N-terminal end is determined by analyzing a highly-purified polypeptide with a gas-phase protein sequencer.

Cloning of the polypeptide gene

In the present invention, a DNA fragment, obtained by separating DNA from a donor microorganism capable of producing the polypeptide and digesting the DNA, for example with ultrasound or restriction enzymes, and a vector fragment, obtained by cleaving a vector in the same way, are ligated, for example with DNA ligase, to obtain a recombinant DNA carrying the polypeptide gene.

The donor microorganism is chosen from bacteria which produce the polypeptide. Examples of such bacteria are those of genus Bacillus such as *Bacillus macerans, Bacillus megaterium, Bacillus circulans, Bacillus polymyxa,* and *Bacillus stearothermophilus,* and those of genus Klebsiella such as *Klebsiella pneumoniae,* as described, for example, in Japan Patent Kokai No. 20,373/72, Japan Patent Kokai No. 63,189/75, Japan Patent Kokai No. 88,290/75, and Hans Bender, *Archives of Microbiology,* Vol.111, pp.271–282 (1977).

Recombinant microorganisms in which polypeptide producibility has been introduced by genetic engineering techniques can also be used as the donor microorganism.

The DNA of the donor microorganism can be prepared by culturing the donor microorganism, for example with a liquid culture medium for about 1–3 days under aeration-agitation conditions, centrifugally collecting the microorganism from the culture, and lysing the microorganism. Examples of bacteriolytic procedures are cytohydrolysis using lysozyme or β-glucanase, and ultrasonic treatment.

Other enzymes, such as protease, and/or surface active agents, such as sodium lauryl sulfate, can be used in combination, if necessary. Of course, freezing-thawing treatment can be carried out, if necessary.

In order to isolate DNA from the resultant lysate, two or more conventional procedures, such as phenol extraction, protein removal, protease treatment, ribonuclease treatment, alcohol sedimentation, and centrifugation, are combined.

Although DNA ligation can be effected by treating DNA- and vector-fragments, for example with ultrasound or restriction enzymes, it is desirable to use restriction enzymes, particularly those acting specifically on a prescribed nucleotide sequence, for smooth ligation. Specifically suited are Type II restriction enzymes, for example, EcoRI, HindIII, BamHI, SalI, SlaI, XmaI, MboI, XbaI, SacI, PstI, etc.

Bacteriophages and plasmids which autonomically proliferate in the host microorganism are suitable for vectors.

When a microorganism of species *Escherichia coli* is used as the host, bacteriophages such as λgt·λC and λgt·λB are employable, while ρ11, ψ1 and ψ105 are usable when a microorganism of species *Bacillus subtilis* is used as the host.

As regards plasmids, when a microorganism of species *Escherichia coli* is used as the host, plasmids such as pBR322 and pBR325 are employable, while pUB110, pTZ4 (pTP4) and pC194 are usable for a host microorganism of species *Bacillus subtilis.* Plasmids which autonomically proliferate in two or more different host microorganisms, for example, pHV14, TRp7, YEp7 and pBS7, can be used as the vector. These vectors are cleaved with the same types of restriction enzymes as used in DNA digestion to obtain a vector fragment.

DNA- and vector-fragments are ligated with conventional procedures using DNA ligase. For example, DNA- and vector-fragments are first annealed, then subjected in vitro to the action of a suitable DNA ligase to obtain a recombinant DNA. If necessary, such recombinant DNA can be prepared by introducing the annealed fragments into the host microorganism to subject them to in vivo DNA ligase.

The host microorganisms usable in the invention are those in which recombinant DNA autonomically and consistently proliferates to express its characteristics. Specifically, microorganisms which are not capable of producing α-amylase (EC 3.2.1.1) are preferably used because the use of such microorganisms facilitates isolation and purification of the secreted polypeptide.

The recombinant DNA can be introduced into the host microorganism with any conventional procedure. For example, when the host microorganism belongs to the species *Escherichia coli,* introduction of recombinant DNA is effected in the presence of calcium ion, while the competent cell- and protoplast-methods are employed when a host microorganism of genus Bacillus is used.

The recombinant microorganism in which recombinant DNA has been introduced is selected by collecting clones which grow on plate culture containing starch to convert the starch into cyclodextrin.

The present inventors found that the recombinant DNA carrying the polypeptide gene cloned in this way can be easily introduced, after isolation from the recombinant microorganism, into a different host microorganism. It was also found that a DNA fragment carrying the polypeptide gene, obtained by digesting a recombinant DNA carrying the gene with restriction enzymes, can be easily ligated with a vector fragment which has been obtained in the same manner.

Furthermore, the present inventors found that the polypeptide gene in the recombinant DNA obtained according to the present invention is cleaved by restriction enzyme PvuII, purchased from Toyobo Co., Ltd., Osaka, Japan, to lose the ability of expressing the polypeptide gene because the recombinant DNA has a PvuII restriction cleavage site.

Sequence of the polypeptide gene

The polypeptide gene is sequenced by the chain-terminator method as described in Gene, Vol.9, pp. 259–268(1982).

This method contains the step of inserting a cloned DNA fragment carrying the polypeptide gene into the insertion site of a suitable plasmid such as pUC18 using restriction enzymes. The obtained recombinant plasmid is introduced by transformation into a suitable *Escherichia coli* strain such as *Escherichia coli* JM83, followed by selection of the recombinant microorganism that contains the plasmid.

The recombinant plasmid is prepared from the proliferated recombinant microorganism.

The obtained recombinant plasmid is annealed together with a synthetic primer, and the Klenow fragment is then allowed to act on the mixture to extend the primer, as well as to form the complementary DNA.

Thereafter, the mixture is subjected sequentially to polyacrylamide-electrophoresis and radioautography, followed by sequencing of the polypeptide gene.

The signal polypeptide which regulates polypeptide secretion from the cell is sequenced in the same manner.

Amino acid sequence of the polypeptide

The amino acid sequence of the polypeptide is determined from the DNA sequence of the polypeptide gene.

The amino acid sequence of the signal peptide is determined in the same manner.

N-terminal amino acid sequence of the polypeptide

A polypeptide producer microorganism of genus Bacillus is cultured with a nutrient culture medium to produce the polypeptide. The supernatant, centrifugally obtained from the culture, is purified by ammonium sulfate fractionation, ion exchange chromatography and high-performance liquid chromatography to obtain a high-purity polypeptide specimen. The specimen is then degraded with a gas-phase protein sequencer in accordance with the method described in *Journal of Biological Chemistry,* Vol. 256, pp. 7990–7997 (1981), and isolated with high-performance liquid chromatography, followed by determination of the partial amino acid sequence of the N terminal end.

Preparation of polypeptide with recombinant microorganism

The present inventors found that a large amount of polypeptide can be consistently produced by culturing a recombinant microorganism with a nutrient culture medium.

To the nutrient culture medium is incorporated, for example, a carbon source, a nitrogen source, minerals, and, if necessary, small amounts of organic nutrients such as amino acids and vitamins.

Starch, partial starch hydrolysate, and saccharides such as glucose, fructose and sucrose are suitable for the carbon source. Inorganic nitrogen sources such as ammonia gas, ammonia water, ammonium salts and nitrates; organic nitrogen sources such as peptone, yeast extract, and defatted soybean, corn steep liquor and meat extract are suitable for the nitrogen source.

The recombinant microorganism is cultured with a nutrient culture medium for about 1–4 days under aeration-agitation conditions to accumulate polypeptide while keeping the culture medium, for example, at pH 4°–10° and 25°–65° C.

Although the polypeptide in the culture may be used intact, generally the culture is separated into polypeptide solution and cells with conventional procedures such as filtration and centrifugation, prior to its use.

When the polypeptide is present in the cells, the cells are first treated with ultrasound, surface active agent and/or cytohydrolysis, then with filtration and centrifugation to separate a solution containing the polypeptide.

The solution containing the polypeptide thus obtained is purified, for example by combination of concentration in vacuo, concentration using a membrane filter, salting-out using ammonium sulfate or sodium sulfate, fractional sedimentation using methanol, ethanol or acetone, to obtain a highly-purified polypeptide specimen which is advantageously usable as industrial polypeptide material.

To further improve the quality of the polypeptide, the amino acid sequence of the polypeptide may be partially substituted, removed, added, or modified in such a manner that the polypeptide does not lose its CGTase activity prior to its use.

One unit of CGTase activity is defined as the amount of polypeptide that diminishes completely the iodine-coloration of 15 mg soluble starch at 40° C. over a period of 10 minutes under the following reaction conditions: To 5 ml of 0.3 w/w % soluble starch solution containing 0.02 M acetate buffer (pH 5.5) and 2×10^{-3} M calcium chloride is added 0.2 ml of a diluted enzyme solution, and the mixture is incubated at 40° C. for 10 minutes. Thereafter, 0.5 ml of the reaction mixture is sampled and 15 ml of 0.02 N aqueous sulfuric acid solution is added to suspend the enzymatic reaction. To the reaction mixture 0.2 ml of 0.1 N I_2-KI solution is added to effect coloration, and its absorbance at a wavelength of 660 nm is determined.

Deposition of recombinant microorganisms

Recombinant microorganisms *Escherichia coli* TCH201, *Escherichia coli* MAH2, *Bacillus subtilis* MAU210, and *Bacillus subtilis* TCU211 have been deposited under the accession numbers of FERM BP-2109, BP-2110, BP-2111, and BP-2112, respectively, at the Fermentation Research Institute, Agency of Industrial Science and Technology, 1–3, Higashi 1 chome, Yatabemachi, Tsukuba-gun, Ibaraki-ken, Japan.

Several embodiments according to the present invention are disclosed in the following examples:

EXAMPLE 1

Cloning of *Bacillus stearothermophilus* polypeptide gene into *Escherichia coli*

Preparation of chromosome DNA carrying the heat-resistant polypeptide gene of *Bacillus stearothermophilus*

The chromosome DNA carrying the heat-resistant-polypeptide gene of *Bacillus stearothermophilus* was prepared in accordance with the method described by Saito and Miura, *Biochimica et Biophisica Acta,* Vol. 72, pp.619–629 (1963). A seed culture of *Bacillus stearothermophilus* FERM-P No. 2225 was cultured with brain heart infusion medium at 50° C. overnight under vigorous shaking conditions. The cells, centrifugally collected from the culture, were suspended with TES buffer (pH 8.0) containing Tris-aminomethane, hydrochloric acid, EDTA and sodium chloride, mixed with 2 mg/ml of lysozyme, and incubated at 37° C. for 30 minutes. The incubated mixture was frozen, allowed to stand at −20° C. overnight, mixed with TSS buffer (pH 9.0) containing Tris-aminomethane, hydrochloric acid, sodium lauryl sulfate and sodium chloride, heated to 60° C., mixed with a mixture of TES buffer (pH 7.5) and phenol (1:4 by volume), cooled in ice-chilled water, and centrifuged to obtain a supernatant. To the supernatant was added two volumes of cold ethanol to recover a crude chromosomal DNA which was then dissolved in SSC buffer (pH 7.1) containing sodium chloride and

trisodium citrate; thereafter, the mixture was subjected to both "RNase A", a ribonuclease commercialized by Sigma Chemical Co., Mo., USA, and "Pronase E", a protease commercialized by Kaken Pharmaceutical Co., Ltd., Tokyo, Japan, mixed with a fresh preparation of TES buffer and phenol mixture, cooled, centrifuged, and mixed with two volumes of cold ethanol to recover a purified chromosomal DNA. The chromosomal DNA was dissolved in a buffer (pH 7.5) containing Tris-aminomethane, hydrochloric acid and EDTA, and stored at −20° C.

EXAMPLE 1-(2)

Preparation of plasmid pBR322

Plasmid pBR322 (ATCC 37013) was isolated from *Escherichia coli* in accordance with the method described by J. Meyer et al. in *Journal of Bacteriology,* Vol. 127, pp. 1524–1537 (1976).

(EXAMPLE 1-(3)

Preparation of recombinant DNA carrying polypeptide gene

The purified chromosomal DNA carrying the heat-resistant-polypeptide gene, prepared in Example 1-(1), was partially digested with restriction enzyme MboI, purchased from Nippon Gene Co., Ltd., Toyama, Japan, to give DNA fragments of 1–20 kbp. Separately, the pBR322 specimen, prepared in Example 1-(2), was completely cleaved with restriction enzyme BamHI, purchased from Nippon Gene Co., Ltd., and the cleaved product was subjected to *Escherichia coli* alkaline phosphatase, purchased from Takara Shuzo Co., Ltd., Kyoto, Japan, to prevent self-ligation of the plasmid fragment as well as to dephosphorize the 5'-terminal end of the fragment.

Both fragments were then ligated by subjecting them to T_4 DNA ligase, purchased from Nippon Gene Co., Ltd., at 4° C. overnight to obtain a recombinant DNA.

EXAMPLE 1-(4)

Introduction of recombinant DNA into *Escherichia coli*

Escherichia coli HB101 (ATCC 33694), a strain incapable of producing amylase, was used as the host.

The microorganism was cultured with L-broth at 37° C. for 4 hours, and the cell, centrifugally collected from the culture, was suspended with 10 mM acetate buffer (pH 5.6) containing 50 mM manganese chloride, centrifugally collected again, resuspended with 10 mM acetate buffer (pH 5.6) containing 125 mM manganese chloride, mixed with the recombinant DNA prepared in Example 1-(3), and allowed to stand in an ice chilled water bath for 30 minutes. The mixture was then warmed to 37° C., mixed with L-broth, spread on L-broth agar plate medium containing 50 μg/ml of ampicillin and 2 mg/ml starch, and incubated at 37° C. for 24 hours to form colonies.

The colonies which had degraded the starch into cyclodextrin were selected by the iodine-coloration method. Thus, the microorganisms in which the recombinant DNA carrying polypeptide gene had been introduced were selected. A recombinant microorganism was then proliferated, and the recombinant DNA was extracted from the proliferated microorganism by the plasmid preparation method in Example 1-(2), subjected to restriction enzymes to determine the restriction cleavage sites, and completely digested with restriction enzyme EcoRI purchased from Nippon Gene Co., Ltd. The digested product was subjected to T_4 DNA ligase similarly as in Example 1-(3) to obtain a recombinant DNA, followed by selection of a recombinant microorganism in accordance with the method in Example 1-(4). The recombinant microorganism contained a recombinant DNA of a relatively small-size that carries no polypeptide gene.

The recombinant DNA and plasmid pBR322 were then completely digested with restriction enzyme SalI, purchased from Nippon Gene Co., Ltd., and treated similarly as in the case of EcoRI to select recombinant microorganisms containing a recombinant DNA of a much smaller-size that carries the polypeptide gene.

One of these microorganisms and its recombinant DNA were named as "Escherichia coli TCH201 (FERM BP-2109)" and "pTCH201".

The restriction map of recombinant DNA pTCH201, in particular that of the DNA fragment derived from *Bacillus stearothermophilus* microorganism, is as shown in FIG. **1**.

FIG. **1** clearly shows that the DNA fragment carrying the polypeptide gene derived from *Bacillus stearothermophilus* microorganism is cleaved by either restriction enzyme PvuII purchased from Toyobo Co., Ltd., KpnI, HindIII purchased from Nippon Gene Co., Ltd., or XbaI purchased from Takara Shuzo Co., Ltd, but not by EcoRI, BamHI, PstI, XhoI, BglII or AccI, all purchased from Nippon Gene Co., Ltd.

EXAMPLE 2

Cloning of polypeptide gene of *Bacillus stearothermophilus* into *Bacillus subtilis*

EXAMPLE 2-(1)

Preparation of recombinant DNA pTCH201

Recombinant DNA pTCH201 was isolated from *Escherichia coli* TCH201 (FERM BP-2109) in accordance with the method in Example 1-(2).

EXAMPLE 2-(2)

Preparation of plasmid pUB110

Plasmid pUB110 (ATCC 37015) was isolated from *Bacillus subtilis* in accordance with the method described by Gryczan et al. in *Journal of Bacteriology,* Vol.134, pp. 318–329 (1978).

EXAMPLE 2-(3)

Preparation of recombinant DNA carrying polypeptide gene

The recombinant DNA pTCH201 carrying the heat-resistant-polypeptide gene prepared in Example 2-(1), was completely digested by subjecting it simultaneously to restriction enzymes EcoRI and XbaI.

Separately, the plasmid pUB110 specimen, prepared in Example 2-(2), was completely cleaved by subjecting it to restriction enzymes EcoRI and XbaI in the same manner.

The resultant fragments were subjected to T_4 DNA ligase similarly as in Example 1-(3) to obtain a recombinant DNA.

EXAMPLE 2-(4)

Introduction of recombinant DNA into *Bacillus subtilis*

In this Example, *Bacillus subtilis* 715A, a strain incapable of producing amylase, was used as the host. The microorganism was cultured with brain heart infusion

medium at 28° C. for 5 hours, and the cell, centrifugally collected from the culture, was then prepared into protoplast suspension in accordance with the method described by Schaeffer et al. in *Proceedings of the National Academy of Sciences of the USA,* Vol.73, pp.2151–2155 (1976).

To the suspension was added the recombinant DNA, prepared in Example 2-(3), and the mixture was then treated in accordance with the method described by Sekiguchi et al. in *Agricultural and Biological Chemistry,* Vol.46, pp.1617–1621 (1982) to effect transformation, spread on HCP medium containing 250 μg/ml of kanamycin and 10 mg/ml of starch, and incubated at 28° C. for 72 hours to form colonies.

From these colonies, recombinant microorganisms in which the recombinant DNA carrying the heat-resistant-polypeptide gene had been introduced were selected by the method in Example 1-(4). One of these microorganisms and its recombinant DNA were named as "*Bacillus subtilis* TCU211 (FERM BP2-2112)" and "pTCU211", respectively.

The restriction map of recombinant DNA pTCU211, in particular that of the DNA fragment derived from *Bacillus stearothermophilus* microorganism, is as shown in FIG. **2**. FIG. **2** clearly shows that the DNA fragment carrying the polypeptide gene derived from *Bacillus stearothermophilus* microorganism is cleaved by either restriction enzyme PvuII, KpnI or HindIII, but not by EcoRI, BamHI, PstI, XhoI, BglII, AccI or XbaI.

EXAMPLE 3

N-terminal amino acid sequence of *Bacillus stearothermophilus* polypeptide

EXAMPLE 3-(1)

Preparation of polypeptide

Bacillus stearothermophilus FERM-P No.2225 was cultured with a liquid culture medium by the method in Example 5 to produce polypeptide. The supernatant, centrifugally obtained from the culture, was salted out with ammonium sulfate to obtain a polypeptide fraction which was then purified by column chromatography using "DEAE Toyopearl 650", an anion exchanger commercialized by Toyo Soda Manufacturing Co., Ltd., Tokyo, Japan, and chromatofocusing using "Mono P", a product of Pharmacia Fine Chemicals AB, Uppsala, Sweden, to obtain a highly-purified polypeptide specimen.

On SDS-polyacrylamide electrophoresis in accordance with the method described by K. Weber and M. Osborn in *Journal of Biological Chemistry,* Vol. 244, page 4406 (1969), the polypeptide specimen showed a molecular weight of 70,000±10,000 daltons.

The specific activity of the polypeptide specimen was 200±30 units/mg protein.

EXAMPLE 3-(2)

N-terminal amino acid sequence of the polypeptide

A polypeptide specimen, prepared by the method in Example 3-(1), was fed to "Model 470A", a gas-phase protein sequencer, a product of Applied Biosystems Inc., Calif., USA, and then analyzed with high-performance liquid chromatography to determine the N-terminal partial amino acid sequence.

The partial amino acid sequence was Ala-Gly-Asn-Leu-Asn-Lys-Val-Asn-Phe-Thr.

EXAMPLE 4

Sequence of polypeptide gene derived from *Bacillus stearothermophilus* and amino acid sequence of polypeptide

EXAMPLE 4-(1)

Preparation of plasmid pUC18

Plasmid pUC18 was prepared in accordance with the method in Example 1-(2) from *Escherichia coli* JM83 (ATCC 35607) in which the plasmid had been introduced.

EXAMPLE 4-(2)

Preparation of recombinant DNA carrying polypeptide gene

The recombinant DNA was prepared by the method in Example 1-(3).

A fragment, obtained by digesting a fragment carrying the polypeptide gene, prepared by the method in Example 2-(3), with restriction enzymes, and a plasmid fragment, obtained by cleaving a pUC18 specimen, prepared by the method in Example 4-(1), in the same manner, were subjected to T_4 DNA ligase to obtain a recombinant DNA.

EXAMPLE 4-(3)

Introduction of recombinant DNA into *Escherichia coli*

In this example, *Escherichia coli* JM83 was used as the host.

The recombinant DNA was introduced into this microorganism in accordance with the method in Example 1-(4) to transform the microorganism.

The recombinant microorganisms were inoculated to a culture medium containing 5-bromo-4-chloro-3-indoyl-β-galactoside (Xgal), and the microorganism forming colorless plaque was selected.

EXAMPLE 4-(4)

Preparation of recombinant DNA from recombinant microorganism

The recombinant microorganism was cultured on L-broth containing 50 μg/ml of ampicillin, and the obtained cells were then treated with the alkaline miniprepartion method to obtain a recombinant DNA.

EXAMPLE 4-(5)

Sequence of recombinant DNA

The recombinant DNA was sequenced by the dideoxy chain terminator method.

The recombinant DNA, prepared in Example 4-(4), and a synthetic primer composed of 17 bases were mixed, annealed at 60° C. for 20 minutes, mixed with dNTP, ddNTP, (α-^{32}P) dCTP and Klenow fragment, and reacted at 37° C. for 30 minutes to extend the primer towards the 3′ site from the 5′ site. Thus, the complementary DNA was obtained. To the complementary DNA was added an excessive amount of dNTP, and the mixture was reacted at 37° C. for 30 minutes, followed by addition of a formamide solution of dye mixture to suspend the reaction. The reaction mixture was boiled for 3 minutes, and electrophoresed on 6% polyacrylamide gel at about 25 mA (about 2,000 volts) to separate the extended complementary DNA. After completion of the electrophoresis, the gel was fixed and dehydrated.

The dehydrated gel was then autographed, and the polypeptide gene was determined by analyzing the base bands on the radioautogram.

The results are as shown in FIG. 5.

The signal peptide gene located upstream of the 5'-terminal end of the polypeptide gene was sequenced in the same manner.

The results are as shown in FIG. 6.

EXAMPLE 4-(6)

Amino acid sequence of the polypeptide

The amino acid sequence of the polypeptide was determined with reference to the sequence as shown in FIG. 5, and the results are as shown in FIG. 8.

The amino acid sequence of the signal peptide was determined in the same manner, and the results are as shown in FIG. 7.

This evidence confirms that the polypeptide derived from *Bacillus stearothermophilus* has the amino acid sequence as shown in FIG. 8.

EXAMPLE 5

Preparation of polypeptide with recombinant microorganism

Polypeptides were prepared with recombinant microorganisms *Escherichia coli* TCH201 (FERM BP-2109) and *Bacillus subtilis* TCU211 (FERM BP-2112) both in which recombinant DNA carrying the heat-resistant-polypeptide gene derived from *Bacillus stearothermophilus* had been introduced.

The polypeptide productivities of these recombinant microorganisms were compared with those of the host microorganisms without the recombinant plasmid and the donor *Bacillus stearothermophilus* microorganism in relation to their CGTase activity. A liquid culture medium consisting of 1.0 w/v % corn steep liquor, 0.1 w/v % ammonium sulfate, 1.0 w/v % calcium carbonate, 1 w/v % starch and water was adjusted to pH 7.2, sterilized by heating at 120° C. for 20 minutes, and cooled. In the case of *Escherichia coli* TCH201, the liquid culture medium was mixed with 50 μg/ml of ampicillin and the microorganism was inoculated to the liquid culture medium. *Escherichia coli* HB101 was inoculated to the liquid culture medium without addition of antibiotic. In each case, the microorganism was cultured at 37° C. for 48 hours under vigorous shaking conditions.

Separately, *Bacillus subtilis* TCU211 was inoculated to the liquid culture medium additionally containing 5 μg/ml of kanamycin, while *Bacillus subtilis* 715A was inoculated to the liquid culture medium without addition of antibiotic. In each case, the microorganism was cultured at 28° C. for 72 hours.

Bacillus stearothermophilus FERM-P No. 2225 was cultured with the liquid culture medium at 50° C. for 48 hours without addition of antibiotic. After separation of each culture into supernatant and cells by centrifugation, the supernatant was assayed intact for CGTase activity, while the cells were ultrasonically broken, prior to determination of their CGTase activity per culture. The results are as shown in Table 1.

TABLE 1

Microorganism	CGTase activity (units/ml) Supernatant	Cell	Total	
Escherichia coli TCH201 (FERM BP-2109)	0.8	13.5	14.3	Present invention
Bacillus subtilis TCU211 (FERM BP-2110)	46.7	20.5	67.2	Present invention
Escherichi coli HB101	0	0	0	Control
Bacillus subtilis 715A	0	0	0	Control
Bacillus stearothermophilus FERM-P No. 2225	8.5	0.3	8.8	Control

This evidence clearly shows that the recombinant microorganisms are advantageously usable in industrial-scale production of polypeptide because these microorganisms possess an improved polypeptide productivity.

The supernatants were salted out with ammonium sulfate at a saturation degree of 0.6 to obtain crude polypeptide specimens. After studying these polypeptide specimens on their enzymatic properties, such as saccharide transfer form starch to sucrose, cyclodextrin production from starch, ratio of α, β- and λ-cyclodextrins, optimum temperature, optimum pH, stable temperature range and stable pH range, the properties of the polypeptide produced by the recombinant microorganism were in good accordance with those of the polypeptide produced by the donor *Bacillus stearothermophilus* microorganism.

EXAMPLE 6

Cloning of *Bacillus macerans* polypeptide gene into *Escherichia coli*

EXAMPLE 6-(1)

Preparation of chromosome DNA carrying *Bacillus macerans* polypeptide gene

The polypeptide gene was prepared in accordance with the method in Example 1-(1), except that *Bacillus macerans* 17A was cultured at 28° C.

EXAMPLE 6-(2)

Preparation of recombinant DNA carrying polypeptide gene

The chromosomal DNA carrying the polypeptide gene derived from *Bacillus macerans*, prepared in Example 6-(1), was partially digested similarly as in Example 1-(3) with restriction enzyme HindIII, purchased from Nippon Gene Co., Ltd.

Separately, a plasmid pBR322 specimen, prepared by the method in Example 1-(2), was completely cleaved with restriction enzyme HindIII, and the 5'-terminal end of the cleaved product was dephosphorized by the method in Example 1 (3). The fragments thus obtained were ligated in accordance with the method in Example 1-(3) to obtain a recombinant DNA.

EXAMPLE 6-(3)

Introduction of recombinant DNA into *Escherichia coli*

The recombinant microorganism in which recombinant DNA had been introduced was cloned in accordance with the method in Example 1-(4) using *Escherichia coli* HB101 (ATCC 33694), a strain incapable of producing amylase, as the host. Thereafter, the recombinant DNA was isolated from the microorganism,

subjected to restriction enzymes to determine the restriction cleavage sites, and partially digested with restriction enzyme Sau3AI commercialized by Nippon Gene Co., Ltd.

Separately, a plasmid pBR322 specimen, obtained by the method in Example 1-(2), was completely cleaved with restriction enzyme BamHI. and the 5'-terminal end of the resultant product was dephosphorized similarly as in Example 1-(3). The obtained fragments were ligated with T_4 DNA ligase to obtain a recombinant DNA, followed by selecting recombinant microorganisms in accordance with the method in Example 1-(4). The recombinant microorganisms contained a recombinant DNA of a relatively small-size that carries the polypeptide gene.

One of these recombinant microorganisms and its recombinant DNA were named as "*Escherichia coli* MAH2 (FERM BP-2110)" and "pMAH2" respectively.

The restriction map of recombinant DNA pMAH2, in particular that of the DNA fragment that carries the polypeptide gene derived from *Bacillus macerans,* is as shown in FIG. **3**.

FIG. **3** shows that the DNA fragment carrying the polypeptide gene derived from *Bacillus macerans* is cleaved by either restriction enzyme PvuII, SalI, AvaI commercialized by Nippon Gene Co., Ltd., or PstI commercialized by Nippon Gene Co., Ltd., but not by EcoRI, HindIII, KcnI, BamHI, XbaI, XhoI or SmaI.

EXAMPLE 7

Cloning of *Bacillus macerans* polypeptide oene into *Bacillus subtilis*

EXAMPLE 7-(1)

Preparation of recombinant DNA pMAH2

The recombinant DNA pMAH2 was isolated from *Escherichia coli* MAH2 (FERM BP-2110) in accordance with the method in Example 1-(2).

EXAMPLE 7-(2)

Preparation of recombinant DNA carrying the polypeptide gene

The recombinant DNA pMAH2 specimen carrying the polypeptide gene, prepared in Example 7-(1), was completely digested by subjecting it simultaneously to restriction enzymes EcoRI and BamHI.

The fragments thus obtained were subjected to T_4 DNA ligase similarly as in Example 1-(3) to obtain a recombinant DNA.

EXAMPLE 7-(3)

Introduction of recombinant DNA into *Bacillus subtilis*

Recombinant microorganisms in which recombinant DNA carrying the polypeptide gene derived from *Bacillus macerans* had been introduced were cloned in accordance with the method in Example 2-(4) using *Bacillus subtilis* 715A, a strain incapable of producing amylase.

One of the recombinant microorganisms and its recombinant DNA were named as "Bacillus subtilis MAU210 (FERM "BP-2111)" and "pMAU210" respectively. The restriction map of recombinant DNA pMAU210, in particular that of the DNA fragment that carries the polypeptide gene derived from Bacillus macerans, was as shown in FIG. **4**. FIG. **4** shows that this DNA fragment carrying the polypeptide gene derived from *Bacillus macerans,* is cleaved by either restriction enzyme PvuII, SalI, AvaI or PstI, but not by EcoRI, HindIII, KpnI, BamHI, XbaI, XhoI or SmaI.

EXAMPLE 8

N-terminal amino acid sequence of the polypeptide derived from *Bacillus macerans*

EXAMPLE 8-(1)

Preparation of polypeptide

The polypeptide was produced by culturing *Bacillus subtilis* MAU210 (FERM BP-2111) with a liquid culture medium similarly as in Example 10 and then purifying in accordance with the method in Example 4-(1) to obtain a high-purity polypeptide specimen.

On SDS polyacrylamide electrophoresis, the polypeptide specimen showed a molecular weight of 70,000±10,000 daltons and a specific activity of 200±30 units/mg protein.

EXAMPLE 8-(2)

N-terminal amino acid sequence

The partial amino acid sequence containing the N-terminal terminal end was determined with the polypeptide specimen prepared in Example 8-(1), in accordance with the method in Example 3-(2).

The partial amino acid sequence was Ser-Pro-Asp-Thr-Ser-Val-Asn-Asn-Lys-Leu.

EXAMPLE 9

Sequence of polypeptide gene derived from *Bacillus macerans* and amino acid sequence of polypeptide

EXAMPLE 9-(1)

Preparation of recombinant DNA carrying the polypeptide gene

The recombinant DNA was prepared in accordance with the method in Example 4-(3).

More particularly, a DNA fragment, obtained by digesting a DNA fragment carrying the polypeptide gene, prepared by the method in Example 7-(2), with restriction enzymes, and a plasmid fragment, obtained by cleaving a plasmid pUCI8 specimen, prepared by the method in Example 4-(2), in the same manner, were ligated with T_4 DNA ligase to obtain a recombinant DNA.

EXAMPLE 9-(2)

Introduction of recombinant DNA into *Escherichia coli*

The recombinant DNA was introduced in accordance with the method in Example 4-(3) into *Escherichia coli* JM83 as the host microorganism to obtain a recombinant microorganism.

EXAMPLE 9-(3)

Preparation of recombinant DNA from recombinant microorganism

The recombinant DNA was prepared in accordance with the method in Example 4-(4).

EXAMPLE 9-(4)

Sequence of recombinant DNA

The polypeptide gene was sequenced in accordance with the method in Example 4-(5).

The results are as shown in FIG. **9**.

The signal peptide located upstream of the 5'-site of the polypeptide gene was sequenced in the same manner.

The results are as shown in FIG. 10.

EXAMPLE 9-(5)

Amino acid sequence of polypeptide

The amino acid sequence of the polypeptide was determined with reference to the sequence of the polypeptide gene. The results are as shown in FIG. **12**.

The amino acid sequence of the signal peptide was determined in the same manner. The results are as shown in FIG. **11**.

This evidence confirms that the polypeptide derived from *Bacillus macerans* has the amino acid sequence as shown in FIG. 12.

The evidence as shown in FIGS. **8** and 12 show that each polypeptide has the following common amino acid sequences:

(a) Asn-Lys-Ile-Asn-Asp-Gly-Tyr-Leu-Thr,

(b) Pro-Val-Phe-Thr-Phe-Gly-Glu-Trp-Phe-Leu,

(c) Val-Thr-Phe-Ile-Asp-Asn-His-Asp-Mct-Asp-Arg-Phe,

(d) Ile-Tyr-Tyr-Gly-Thr-Glu-Gln-Tyr-Met-Thr-Gly-Asn-Gly-Asp-Pro-Asn-Asn-Arg, and

(e) Asn-Pro-Ala-Leu-Ala-Tyr-Gly,

as well as that these partial amino acid sequences (a), (b), (c), (d) and (e) are located in order of nearness to the N-terminal end of the polypeptide. These common sequences are underlined in FIGS. 8 and 12.

EXAMPLE 10

Preparation of polypeptide with recombinant microorganism

Polypeptides were prepared with *Escherichia coli* MAH2 (FERM BP-2110) and *Bacillus subtilis* MAU210 (FERM BP-2111) both in which recombinant DNA carrying the polypeptide gene derived from *Bacillus macerans* had been introduced. The polypeptide productivities of these recombinant microorganisms, the host microorganisms without addition of the recombinant plasmid, and the donor *Bacillus macerans* microorganism were compared in relation to their CGTase activity. A liquid culture medium prepared by the method in Example 5 was used.

Escherichia coli MAH2 was inoculated to the liquid culture medium additionally containing 50 μg/ml of ampicillin, while *Escherichia coli* HB101 was inoculated to the liquid culture medium without addition of antibiotic. In each case, the microorganism was cultured at 35° C. for 24 hours under vigorous shaking conditions.

Bacillus subtilis MAU210 was inoculated to the liquid culture medium additionally containing 5 μg/ml of kanamycin, while *Bacillus subtilis* 715A was inoculated to the liquid culture medium without addition of antibiotic. In each case, microorganism was cultured at 28° C. for 72 hours.

Bacillus macerans 17A was cultured with the liquid culture medium at 28° C. for 72 hours without addition of antibiotic.

Each culture was treated similarly as in Example 5, and its CTGase activity was then determined. The results are as shown in Table 2.

TABLE 2

Microorganism	CGTase activity (units/ml) Supernatant	Cell	Total	
Escherichia coli MAH2 (FERM P-7925)	0.6	11.8	12.4	Present invention
Bacillus subtilis MAU210 (FERM P-7926)	54.6	0.3	54.9	Present invention
Escherichi coli HB101	0	0	0	Control
Bacillus subtilis 715A	0	0	0	Control
Bacillus macerans 17A	7.5	0.4	7.9	Control

This evidence clearly shows that the recombinant microorganisms are advantageously usable in industrial-scale production of polypeptide because they have an improved polypeptide productivity.

The supernatants were salted out with ammonium sulfate at a saturation degree of 0.6 to obtain crude polypeptide specimens.

On studying these crude polypeptide specimens on their enzymatic properties similarly as in Example 5, the enzymatic properties of the polypeptide produced by the recombinant microorganisms were in good accordance with those of the polypeptide produced by the donor *Bacillus macerans* microorganism.

Principal uses of the polypeptide will hereinafter be described.

The polypeptide effects the intra- or intermolecular saccharide transfer reaction between suitable saccharide donor and saccharide acceptor.

According to one aspect of the present invention, various saccharide-transferred products can be produced by taking advantage of these saccharide transfer reactions.

For example, a partial starch hydrolysate containing α-, β- and γ-cyclodextrins is prepared by subjecting an amylaceous substance as the substrate, such as starch, liquefied starch with a Dextrose Equivalent (DE) of below 10, or amylose, to the action of the polypeptide utilizing the intramolecular saccharide transfer reaction. Each cyclodextrin can be isolated from the partial starch hydrolysate, if necessary.

α-Glycosylated saccharide sweetener, for example, α-glycosyl-, α-maltosyl- and α-maltotriosyl-saccharides, is prepared by subjecting a mixture of a saccharide donor, for example, amylaceous substance such as starch, liquefied starch, dextrin, cyclodextrin or amylose; and a saccharide acceptor, for example, monosaccharide such as xylose, sorbose or fructose, or disaccharide such as sucrose, maltulose or isomaltulose, to the action of polypeptide utilizing the intermolecular saccharide transfer action. The α-glycosylated saccharide sweetener can be advantageously used in foods and beverages because the α-glycosylated saccharide sweetener is much milder in taste, more soluble in water, but less crystallizable in comparison with intact saccharide sweetener. These would expand extremely the use of saccharide sweeteners.

In the intermolecular saccharide transfer reaction, the use of a glycoside, for example, steviol glycoside such as stevioside or rebaudioside, glycyrrhizin,

soyasaponin, teasaponin, rutin or esculin, as the saccharide acceptor leads to the formation of α-glycosylated glycosides such as α-glucosyl-, α-maltosyl- and α-maltotriosyl-glycosides. The α-glycosylated glycoside is free of the unpleasant tastes such as bitter- and astringent-tastes which are inherent to intact glycoside, and more readily soluble in water than intact glycoside. These would expand extremely the use of glycosides. Specifically, α-glycosylated steviol glycoside and α-glycosylated glycyrrhizin can be advantageously used in foods, beverages, and pharmaceuticals for peroral administration because the taste improvement in these α-glycosylated glycosides is remarkably high, as well as because their sweetness is comparable to that of sucrose.

Several embodiments will be disclosed.

EXAMPLE 11

Corn syrup containing cyclodextrin

A 10 w/w % suspension of potato starch was mixed with 2 units/g starch of a polypeptide specimen prepared with *Bacillus subtilis* TCU211 in accordance with the method in Example 5, liquefied by heating to 85° C. at pH 6.5, cooled to 70° C., further mixed with the same amount of the polypeptide specimen, and reacted for 40 hours. The reaction mixture was purified by decoloration using activated carbon and deionization using ion exchange resin, and then concentrated to obtain a starch syrup containing cyclodextrin in a yield of 92% based on the dry solid. The corn syrup can be advantageously incorporated into flavors and cosmetics wherein fragrance or aroma is one of the important factors because the corn syrup is excellent in flavor-locking properties.

The α-, β- and γ-cyclodextrins in the corn syrup can be separated by treating it with a procedure using organic precipitant, such as toluene or trichloromethane, or conventional column chromatography.

EXAMPLE 12

α-Glycosylsucrose

A 35 w/w % suspension of cornstarch was mixed with 0.2 w/w % oxalic acid, autoclaved to 120° C. to give a DE of 20, neutralized with calcium carbonate, and filtered to obtain a dextrin solution. The dextrin solution was then mixed with a half amount of sucrose based on the dry solid, and the resultant mixture was mixed with 15 units/g starch of a polypeptide specimen prepared with *Bacillus subtilis* MAU210 in accordance with the method in Example 10, and reacted at pH 6.0 and 55° C. for 40 hours. The reaction mixture was purified by decoloration using activated carbon and deionization using ion exchange resin, and then concentrated to obtain a colorless, transparent corn syrup in a yield of 94% based on the dry solid. The corn syrup containing a large amount of α-glycosylsucrose can be advantageously used in confectioneries because it is mildly sweet and amorphous.

EXAMPLE 13

α-Glycosyl stevioside

Two-hundred g of stevioside and 600 g of dextrin (DE 8) were dissolved in 3 liters of water by heating, and the resultant solution was cooled to 70° C., mixed with 5 units/g dextrin of a polypeptide specimen prepared with *Bacillus subtilis* TCU211 in accordance with the method in Example 5, and reacted at pH 6.0 and 65° C. for 35 hours. The reaction mixture was then heated to 95° C. for 15 minutes, purified by filtration, concentrated, and pulverized to obtain a pulverulent sweetener containing α-glycosyl stevioside in a yield of about 92% based on the dry solid.

The sweetener, free of the unpleasant taste which is inherent to intact stevioside, was comparable to sucrose in taste quality, and the sweetening power of the sweetener was about 100-fold higher than that of sucrose. The sweetener can be advantageously used as a diet sweetener or to season foods and beverages because of its low-cariogenic and low-calorific properties.

EXAMPLE 14

α-Glycosyl ginsenoside

Sixty g of a ginseng extract and 180 g of β-cyclodextrin were dissolved in 500 ml of water by heating, and the resultant mixture was cooled to 70° C., adjusted to pH 6.0, mixed with 3 units/g β-cyclodextrin of a polypeptide specimen prepared with *Escherichia coli* TCH201 in accordance with the method in Example 5, cooled to 65° C., and reacted to pH 6.0 for 40 hours. The reaction mixture was heated for 15 minutes to inactivate the polypeptide, followed by filtration. The filtrate was admitted to a column packed with 3 liters of "Amberlite XAD-7", a synthetic adsorbent commercialized by Rohm & Haas Co., Philadelphia, Pa., USA; thereafter, the column was sufficiently washed with water to remove free saccharides. To the column was then admitted 10 liters of 50 v/v % ethanol, and the eluate was concentrated and dehydrated to obtain about 21 g of a pulverulent product that contains α-glycosyl ginsenoside. Since the product is free of the unpleasant tastes such as bitter-, astringent- and harsh-tastes which are inherent to intact ginsenoside, the product can be perorally administered intact, or, if necessary, seasoned with any sweetener or sour, prior to its use. In addition, the product can be advantageously used in health foods and medicines for internal administration because the product possesses invigorating, peptic, intestine-regulating, haematic, anti-inflammatory and expectorant effects as intact ginsenoside does.

As described above, the present inventors determined the sequences of the CGTase polypeptide gene and its signal peptide, and prepared the recombinant DNA having a PvuII restriction site from a donor microorganism by in vitro genetic engineering techniques. Furthermore, the present inventors prepared recombinant microorganisms in which the recombinant DNA is introduced, as well as confirming that the recombinant microorganisms autonomically and consistently proliferate in a nutrient culture medium.

In view of adequately supplying polypeptide, the present invention is industrially significant because the present invention assures a wide polypeptide source and easily improves the polypeptide productivity of donor microorganisms.

While there has been described what is at present considered to be the preferred embodiments of the invention, it will be understood that various modifications may be made therein, and it is intended to cover in the appended claims all such modifications as fall within the true spirit and scope of the invention.

We claim:

1. A polypeptide possessing cyclomaltodextrin glucanotransferase (CGTase) activity, comprising one or more partial amino acid sequences selected from the group consising of

(a) Asn-Lys-Ile-Asn-Asp-Gly-Tyr-Leu-Thr,
(b) Pro-Val-Phe-Thr-Phe-Gly-Glu-Tri-Phe-Leu,
(c) Val-Thr-Phe-Ile-Asp-Asn-His-Asp-Met-Asp-Arg-Phe,
(d) Ile-Tyr-Thr-Gly-Thr-Glu-Gin-Tyr-Met-Thr-Gly-Asn-Gly-Asp-Pro-Asn-Asn-Arg, and
(e) Asn-Pro-Ala-Leu-Ala-Tyr-Gly.

2. The polypeptide in accordance with claim **1**, wherein said partial amino acid sequences of
(a) Asn-Lys-Ile-Asn-Asp-Gly-Tyr-Leu-Thr,
(b) Pro-Val-Phe-Thr-Phe-Gly-Glu-Trp-Phe-Leu,
(c) Val-Thr-Phe-Ile-Asp-Asn-His-Asp-Met-Asp-Arg-Phe,
(d) Ile-Tyr-Tyr-Gly-Thr-Glu-Gln-Tyr-Met-Thr-Gly-Asn-Gly-Asp-Pro-Asn-Asn-Arg, and
(e) Asn-Pro-Ala-Leu-Ala-Tyr-Gly
are located in order or relative nearness to the N-terminal end of said polypeptide, such that (a) is nearer to the N-terminal end than (b), (b) is nearer than (c), (c) is nearer than (d) and (d) is nearer than (e).

3. The polypeptide in accordance with claim **1**, which shows a molecular weight of 70,000±10,000 daltons on SDS-polyacrylamide electrophoresis.

4. The polypeptide in accordance with claim **1**, whose N-terminal sequence is Ala-Gly-Asn-Leu-Asn-Lrs-Val-Asn-Phe-Thr.

5. The polypeptide in accordance with claim **4**, which has the following amino acid sequence:

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1>	Ala	Gly	Asn	Leu	Asn	Lys	Val	Asn	Phe	Thr	Ser	Asp	Val	Val	Tyr
16>	Gln	Ile	Val	Val	Asp	Arg	Phe	Val	Asp	Gly	Asn	Thr	Ser	Asn	Asn
31>	Pro	Ser	Gly	Ala	Leu	Phe	Ser	Ser	Gly	Cys	Thr	Asn	Leu	Arg	Lys
46>	Tyr	Cys	Gly	Gly	Asp	Trp	Gln	Gly	Ile	Ile	Asn	Lys	Ile	Asn	Asp
61>	Gly	Tyr	Leu	Thr	Asp	Met	Gly	Val	Thr	Ala	Ile	Trp	Ile	Ser	Gln
76>	Pro	Val	Glu	Asn	Val	Phe	Ser	Val	Met	Asn	Asp	Ala	Ser	Gly	Ser
91>	Ala	Ser	Tyr	His	Gly	Tyr	Trp	Ala	Arg	Asp	Phe	Lys	Lys	Pro	Asn
106>	Pro	Phe	Phe	Gly	Thr	Leu	Ser	Asp	Phe	Gln	Arg	Leu	Val	Asp	Ala
121>	Ala	His	Ala	Lys	Gly	Ile	Lys	Val	Ile	Ile	Asp	Phe	Ala	Pro	Asn
136>	His	Thr	Ser	Pro	Ala	Ser	Glu	Thr	Asn	Pro	Ser	Tyr	Met	Glu	Asn
151>	Gly	Arg	Leu	Tyr	Asp	Asn	Gly	Thr	Leu	Leu	Gly	Gly	Tyr	Thr	Asn
166>	Asp	Ala	Asn	Met	Tyr	Phe	His	His	Asn	Gly	Gly	Thr	Thr	Phe	Ser
181>	Ser	Leu	Glu	Asp	Gly	Ile	Tyr	Arg	Asn	Leu	Phe	Asp	Leu	Ala	Asp
196>	Leu	Asn	His	Gln	Asn	Pro	Val	Ile	Asp	Arg	Tyr	Leu	Lys	Asp	Ala
211>	Val	Lys	Met	Trp	Ile	Asp	Met	Gly	Ile	Asp	Gly	Ile	Arg	Met	Asp
226>	Ala	Val	Lys	His	Met	Pro	Phe	Gly	Trp	Gln	Lys	Ser	Leu	Met	Asp
241>	Glu	Ile	Asp	Asn	Tyr	Arg	Pro	Val	Phe	Thr	Phe	Gly	Glu	Trp	Phe
256>	Leu	Ser	Glu	Asn	Glu	Val	Asp	Ala	Asn	Asn	His	Tyr	Phe	Ala	Asn
271>	Glu	Ser	Gly	Met	Ser	Leu	Leu	Asp	Phe	Arg	Phe	Gly	Gln	Lys	Leu
286>	Arg	Gln	Val	Leu	Arg	Asn	Asn	Ser	Asp	Asn	Trp	Tyr	Gly	Phe	Asn
301>	Gln	Met	Ile	Gln	Asp	Thr	Ala	Ser	Ala	Tyr	Asp	Glu	Val	Leu	Asp
316>	Gln	Val	Thr	Phe	Ile	Asp	Asn	His	Asp	Met	Asp	Arg	Phe	Met	Ile
331>	Asp	Gly	Gly	Asp	Pro	Arg	Lys	Val	Asp	Met	Ala	Leu	Ala	Val	Leu
346>	Leu	Thr	Ser	Arg	Gly	Val	Pro	Asn	Ile	Tyr	Tyr	Gly	Thr	Glu	Gln
361>	Tyr	Met	Thr	Gly	Asn	Gly	Asp	Pro	Asn	Asn	Arg	Lys	Met	Met	Ser
376>	Ser	Phe	Asn	Lys	Asn	Thr	Arg	Ala	Tyr	Gln	Val	Ile	Gln	Lys	Leu
391>	Ser	Ser	Leu	Arg	Arg	Asn	Asn	Pro	Ala	Leu	Ala	Tyr	Gly	Asp	Thr
406>	Glu	Gln	Arg	Trp	Ile	Asn	Gly	Asp	Val	Tyr	Val	Tyr	Glu	Arg	Gln
421>	Phe	Gly	Lys	Asp	Val	Val	Leu	Val	Arg	Val	Asn	Arg	Ser	Ser	Ser
436>	Ser	Asn	Tyr	Ser	Ile	Thr	Gly	Leu	Phe	Thr	Ala	Leu	Pro	Ala	Gly
451>	Thr	Tyr	Thr	Asp	Gln	Leu	Gly	Gly	Leu	Leu	Asp	Gly	Asn	Thr	Ile
466>	Gln	Val	Gly	Ser	Asn	Gly	Ser	Val	Asn	Ala	Phe	Asp	Leu	Gly	Pro
481>	Gly	Glu	Val	Gly	Val	Trp	Ala	Tyr	Ser	Ala	Thr	Glu	Ser	Thr	Pro
496>	Ile	Ile	Gly	His	Val	Gly	Pro	Met	Met	Gly	Gln	Val	Gly	His	Gln
511>	Val	Thr	Ile	Asp	Gly	Glu	Gly	Phe	Gly	Thr	Asn	Thr	Gly	Thr	Val
526>	Lys	Phe	Gly	Thr	Thr	Ala	Ala	Asn	Val	Val	Ser	Trp	Ser	Asn	Asn
541>	Gln	Ile	Val	Val	Ala	Val	Pro	Asn	Val	Ser	Pro	Gly	Lys	Tyr	Asn
556>	Ile	Thr	Val	Gln	Ser	Ser	Ser	Gly	Gln	Thr	Ser	Ala	Ala	Tyr	Asp
571>	Asn	Phe	Glu	Val	Leu	Thr	Asn	Asp	Gln	Val	Ser	Val	Arg	Phe	Val
586>	Val	Asn	Asn	Ala	Thr	Thr	Asn	Leu	Gly	Gln	Asn	Ile	Tyr	Ile	Val
601>	Gly	Asn	Val	Tyr	Glu	Leu	Gly	Asn	Trp	Asp	Thr	Ser	Lys	Ala	Ile
616>	Gly	Pro	Met	Phe	Asn	Gln	Val	Val	Tyr	Ser	Tyr	Pro	Thr	Trp	Tyr
631>	Ile	Asp	Val	Ser	Val	Pro	Glu	Gly	Lys	Thr	Ile	Glu	Phe	Lys	Phe
646>	Ile	Lys	Lys	Asp	Ser	Gln	Gly	Asn	Val	Thr	Trp	Glu	Ser	Gly	Ser
661>	Asn	His	Val	Tyr	Thr	Thr	Pro	Thr	Asn	Thr	Thr	Gly	Lys	Ile	Ile
676>	Val	Asp	Trp	Gln	Asn										

6. The polypeptide in accordance with claim **4**, wherein a signal peptide having an amino acid sequence of Met-Arg-Arg-Trp-Leu-Ser-Leu-Val-Leu-Ser-Met-Ser-Phe-Val-Phe-Ser-Ala-Ile-Phe-Ile-Val-Ser-Asp-Thr-Gln-Lys-Val-Thr-Val-Glu-Ala is located upstream at the N-terminal side of said polypeptide.

7. The polypeptide in accordance with claim **1**, whose N-terminal sequence is Ser-Pro-Asp-Thr-Ser-Val-Asn-Asn-Lys-Leu.

8. The polypeptide in accordance with claim **1**, which has the following amino acid sequence;

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1>	Ser	Pro	Asp	Thr	Ser	Val	Asn	Asn	Lys	Leu	Asn	Phe	Ser	Thr	Asp
16>	Thr	Val	Tyr	Gln	Ile	Val	Thr	Asp	Arg	Phe	Val	Asp	Gly	Asn	Ser
31>	Ala	Asn	Asn	Pro	Thr	Gly	Ala	Ala	Phe	Ser	Ser	Asp	His	Ser	Asn
46>	Leu	Lys	Leu	Tyr	Phe	Gly	Gly	Asp	Trp	Gln	Gly	Ile	Thr	Asn	Lys

-continued

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
61>	Ile	Asn	Asp	Gly	Tyr	Leu	Thr	Gly	Met	Gly	Ile	Thr	Ala	Leu	Trp
76>	Ile	Ser	Gln	Pro	Val	Gly	Asn	Ile	Thr	Ala	Val	Ile	Asn	Tyr	Ser
91>	Gly	Val	Asn	Asn	Thr	Ala	Tyr	His	Gly	Tyr	Trp	Pro	Arg	Asp	Phe
106>	Lys	Lys	Thr	Asn	Ala	Ala	Phe	Gly	Ser	Phe	Thr	Asp	Phe	Ser	Asn
121>	Leu	Ile	Ala	Ala	Ala	His	Ser	His	Asn	Ile	Lys	Val	Val	Met	Asp
136>	Phe	Ala	Pro	Asn	His	Thr	Asn	Pro	Ala	Ser	Ser	Thr	Asp	Pro	Ser
151>	Phe	Ala	Glu	Asn	Gly	Ala	Leu	Tyr	Asn	Asn	Gly	Thr	Leu	Leu	Gly
166>	Lys	Tyr	Ser	Asn	Asp	Thr	Ala	Gly	Leu	Phe	His	His	Asn	Gly	Gly
181>	Thr	Asp	Phe	Ser	Thr	Thr	Glu	Ser	Gly	Ile	Tyr	Lys	Asn	Leu	Tyr
196>	Asp	Leu	Ala	Asp	Ile	Asn	Gln	Asn	Asn	Asn	Thr	Ile	Asp	Ser	Tyr
211>	Leu	Lys	Glu	Ser	Ile	Gln	Leu	Trp	Leu	Asn	Leu	Gly	Val	Asp	Gly
226>	Ile	Arg	Phe	Asp	Ala	Val	Lys	His	Met	Pro	Gln	Gly	Trp	Gln	Lys
241>	Ser	Tyr	Val	Ser	Ser	Ile	Tyr	Ser	Ser	Ala	Asn	Pro	Val	Phe	Thr
256>	Phe	Gly	Glu	Trp	Phe	Leu	Gly	Pro	Asp	Glu	Met	Thr	Gln	Asp	Asn
271>	Ile	Asn	Phe	Ala	Asn	Gln	Ser	Gly	Met	His	Leu	Leu	Asp	Phe	Ala
286>	Phe	Ala	Gln	Glu	Ile	Arg	Glu	Val	Phe	Arg	Asp	Lys	Ser	Glu	Thr
301>	Met	Thr	Asp	Leu	Asn	Ser	Val	Ile	Ser	Ser	Thr	Gly	Ser	Ser	Tyr
316>	Asn	Tyr	Ile	Asn	Asn	Met	Val	Thr	Phe	Ile	Asp	Asn	His	Asp	Met
331>	Asp	Arg	Phe	Gln	Gln	Ala	Gly	Ala	Ser	Thr	Arg	Pro	Thr	Glu	Gln
346>	Ala	Leu	Ala	Val	Thr	Leu	Thr	Ser	Arg	Gly	Val	Pro	Ala	Ile	Tyr
361>	Tyr	Gly	Thr	Glu	Gln	Tyr	Met	Thr	Gly	Asn	Gly	Asp	Pro	Asn	Asn
376>	Arg	Gly	Met	Met	Thr	Gly	Phe	Asp	Thr	Asn	Lys	Thr	Ala	Tyr	Lys
391>	Val	Ile	Lys	Ala	Leu	Ala	Pro	Leu	Arg	Lys	Ser	Asn	Pro	Ala	Leu
406>	Ala	Tyr	Gly	Ser	Thr	Thr	Gln	Arg	Trp	Val	Asn	Ser	Asp	Val	Tyr
421>	Val	Tyr	Glu	Arg	Lys	Phe	Gly	Ser	Asn	Val	Ala	Leu	Val	Ala	Val
436>	Asn	Arg	Ser	Ser	Thr	Thr	Ala	Tyr	Pro	Ile	Ser	Gly	Ala	Leu	Thr
451>	Ala	Leu	Pro	Asn	Gly	Thr	Tyr	Thr	Asp	Val	Leu	Gly	Gly	Leu	Leu
466>	Asn	Gly	Asn	Ser	Ile	Thr	Val	Asn	Gly	Gly	Thr	Val	Ser	Asn	Phe
481>	Thr	Leu	Ala	Ala	Gly	Gly	Thr	Ala	Val	Trp	Gln	Tyr	Thr	Thr	Thr
496>	Glu	Ser	Ser	Pro	Ile	Ile	Gly	Asn	Val	Gly	Pro	Thr	Met	Gly	Lys
511>	Pro	Gly	Asn	Thr	Ile	Thr	Ile	Asp	Gly	Arg	Gly	Phe	Gly	Thr	Thr
526>	Lys	Asn	Lys	Val	Thr	Phe	Gly	Thr	Thr	Ala	Val	Thr	Gly	Ala	Asn
541>	Ile	Val	Ser	Trp	Glu	Asp	Thr	Glu	Ile	Lys	Val	Lys	Val	Pro	Asn
556>	Val	Ala	Ala	Gly	Asn	Thr	Ala	Val	Thr	Val	Thr	Asn	Ala	Ala	Gly
571>	Thr	Thr	Ser	Ala	Ala	Phe	Asn	Asn	Phe	Asn	Val	Leu	Thr	Ala	Asp
586>	Gln	Val	Thr	Val	Arg	Phe	Lys	Val	Asn	Asn	Ala	Thr	Thr	Ala	Leu
601>	Gly	Gln	Asn	Val	Tyr	Leu	Thr	Gly	Asn	Val	Ala	Glu	Leu	Gly	Asn
616>	Trp	Thr	Ala	Ala	Asn	Ala	Ile	Gly	Pro	Met	Tyr	Asn	Gln	Val	Glu
631>	Ala	Ser	Tyr	Pro	Thr	Trp	Tyr	Phe	Asp	Val	Ser	Val	Pro	Ala	Asn
646>	Thr	Ala	Leu	Gln	Phe	Lys	Phe	Ile	Lys	Val	Asn	Gly	Ser	Thr	Val
661>	Thr	Trp	Glu	Gly	Gly	Asn	Asn	His	Thr	Phe	Thr	Ser	Pro	Ser	Ser
676>	Gly	Val	Ala	Thr	Val	Thr	Val	Asp	Trp	Gln	Asn				

9. The polypeptide in accordance with claim **8**, wherein a signal peptide having an amino acid sequence of Met-Lys-Lys-Gln-Val-Lys-Trp-Leu-Thr-Ser-Val-Ser-Met-Ser-Val-Gly-Ile-Ala-Leu-Gly-Ala-Ala-Leu-Pro-Val-Trp-Ala is located upstream at the N-terminal side of said polypeptide.

10. The polypeptide in accordance with claim **1**, produced by a microorganism of species *Bacillus stearothermophilus.*

11. The polypeptide in accordance with claim **1**, produced by a microorganism of species *Bacillus macerans.*

12. The polypeptide in accordance with claim **1**, produced by a recombinant microorganism in which a recombinant DNA carrying CGTase gene has been introduced.

13. A process for producing a polypeptide in accordance with claim **1**, comprising:

culturing with a nutrient culture medium a recombinant microorganism having a recombinant DNA carrying isolated structural and promoter genes coding for the expression of said polypeptide; and

recovering the accumulated polypeptide.

14. The process in accordance with claim **13**, wherein said recombinant microorganism is of the genus Escherichia or Bacillus.

15. The process in accordance with claim **13**, wherein said recombinant microorganism is a member selected from the group consisting of *Escherichia coli* TCH201 (FERM BP-2109) or *Escherichia coli* MAH2 (FERM BP-2110).

16. The process in accordance with claim **13**, wherein said recombinant microoroganism is a member selected from the group consisting of *Bacillus subtilis* MAU210 (FERM BP-2111) and *Bacillus subtilis* TCU211 (FERM BP-2112).

17. DNA consisting essentially of DNA coding for a polypeptide possessing cyclomaltodextrin glucanotransferase (CGTase) activity, comprising one or more partial amino acid sequences selected from the group consisting of

(a) Asn-Lys-Ile-Asn-Asp-Gly-Tyr-Leu-Thr,

(b) Pro-Val-Phe-Thr-Phe-Gly-Glu-Trp-Phe-Leu,

(c) Val-Thr-Phe-Ile-Asp-Asn-His-Asp-Met-Asp-Arg-Phe,

(d) Ile-Tyr-Tyr-Gly-Thr-Glu-Gln-Tyr-Met-Thr-Gly-Asn-Gly-Asp-Pro-Asn-Asn-Arg, and

(e) Asn-Pro-Ala-Leu-Ala-Tyr-Gly.

18. DNA consisting essentially of DNA coding for the polypeptide in accordance with claim **5**.

19. DNA consisting essentially of DNA coding for the polypeptide in accordance with claim **8**.

20. DNA in accordance with claim **17**, wherein said DNA is recombinant DNA carrying isolated structural and promoter genes coding for the expression of said polypeptide, wherein said structural and promoter genes have been isolated from a donor microorganism of the genus Bacillus.

21. The recombinant DNA in accordance with claim **20**, wherein said donor microorganism is of the species *Bacillus stearothermophilus.*

22. A biologically-pure culture of a recombinant microorganism having a recombinant DNA which includes DNA in accordance with claim 17.

23. The culture in accordance with claim 22, wherein said recombinant microorganism is of genus Escherichia or Bacillus.

24. The culture in accordance with claim 22, wherein said recombinant microorganism is a member selected from the group consisting of *Escherichia coli* TCH201 (FERM BP-2109) or *Escherichia coli* MAH2 (FERM BP-2110).

25. The culture in accordance with claim 22, wherein said recombinant microorganism is a member selected from the group consisting of *Bacillus subtilis* MAU210 (FERM BP-2111) and *Bacillus subtilis* TCU211 (FERM BP-2112).

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